KOREAN JOURNAL OF ZOOLOGY
Vol. 30, No. 3, 239-247 (1987)

Ultrastructural Study on Antitoxic Effect of Red Ginseng Extract against Toxicity of Methylmercury Chloride in Mouse Kidney

Hee Won Chung and Choon Koo Lee
(Dept. of Biology, Sookmyung Women's University)

생쥐의 腎臟에 미치는 염화 메틸水銀 毒性에 대한 紅蔘抽出物의 抗毒性的 影響에 관한 微細構造的 硏究

鄭 喜 媛·李 春 九
(淑明女子大學校 生物學科)

(Received April 27, 1987)

要 約

생쥐 腎臟의 近位細尿管과 遠位細尿管 細胞의 微細構造에 미치는 염화 메틸水銀의 毒性에 대한 紅蔘抽出物의 抗毒性的 影響을 電子顯微鏡的으로 연구하였다. 염화 메틸水銀 處理群의 近位細尿管 細胞에서는 對照群에서 보다 微細絨毛가 다소 축소되고 불규칙한 배열을 하였다. Brush border의 인접 부위에서는 작은 腔胞들이 나타나고 細胞質 中央 部位에서는 緻密體를 함유한 큰 腔胞들이 觀察되었다. Mitochondria는 상당히 팽대되고 基底膜은 부분적으로 비후되었으며 다수의 lysosome이 나타났다.

염화 메틸水銀 處理群의 遠位細尿管 細胞에서는 불규칙한 細胞 表面, 壞死된 細胞, 그리고 다수의 ribosome과 소수의 脂肪滴이 나타났으며 mitochondria는 팽대되고 基底膜은 부분적으로 비후되었다.

염화 메틸水銀—紅蔘抽出物 併行 處理群의 近位細尿管 細胞에서는 염화 메틸水銀 處理群에서 보다 mitochondria의 팽대 정도가 감소되었으며 腔胞의 크기와 수도 상당히 감소되었다.

염화 메틸水銀—紅蔘抽出物 併行 處理群의 遠位細尿管 細胞에서는 細胞 表面이 어느정도 규칙적이고 mitochondria의 팽대와 基底膜의 비후 정도가 감소되어 正常 細胞와 거의 유사하였다.

INTRODUCTION

Mercury binds to sulfhydryl groups of membrane protein and then causes the inactivation of membrane ATPase and the blockage of glucose transport into the cell. Mercury is con-

centrated and eliminated by the kidney which is particularly susceptible to its toxic action (Hidgson and Guthrie, 1982). Methylated mercury is combined with hemoglobin in the erythrocytes and circulates in this form (Guthrie and Perry, 1980; Arena, 1986). This methylated mercury penetrates cell membranes much more readily than inorganic mercury because of its lipid solubility. For example, the degree of penetration of methyl mercuric chloride is 20 times more potent than that of mercuric chloride (Fang and Fallin, 1976). Methyl mercury is distributed mainly to the liver, kidney, and central nervous system, and has toxic influences on these organs (Donaldson and Gubler, 1978). The accumulation of large quantities of methyl mercury induces morphologic and functional alterations in these organs (Valek, 1965; Ware *et al.*, 1974).

Meanwhile, ginseng, *Panax ginseng*, extract is absorbed rapidly in the gastrointestinal tract and distributed to the liver, kidney, brain, stomach, and long (Han, 1974). Brekhman and Dardymov (1969) reported adaptogenic theory that ginseng increases resistance of living body under adverse conditions such as physical and chemical stresses nonspecifically. Ginseng promotes synthesis of protein and can prevent the progression of pathological and ultrastructural changes (Bae, 1978). It also has the detoxifying effect for toxic materials in the tissues by reducing the accumulation of toxic materials (Hong *et al.*, 1979). However, little is known about the precise mechanisms on detoxifying actions of ginseng in animals.

In this paper, the antitoxic effect of red ginseng extract against toxicity of methylmercury chloride to fine structures of proximal and distal tubule cells of mouse kidney was studied by electron microscopy.

MATERIALS AND METHODS

Thirty female ICR mice, 20~30g, were divided into three groups of control, group I, and group II of ten animals each. The animals were housed in cages with food and water.

Control group was administered orally with distilled water for 23 days. Group I was treated with methylmercury chloride for 23 days. Group II was treated with methylmercury chloride and red ginseng extract for 23 days after pretreatment of red ginseng extract for 7 days. The dose of methylmercury chloride was 15mg/kg b.w./day and that of red ginseng extract was 200mg/kg b.w./day. The red ginseng extract containing crude saponin of 150 mg/g was obtained from the Korean Office of Monopoly.

The kidney taken from the mouse for electron microscopic observation was diced into small cubes, immediately fixed in 2% paraformaldehyde-2.5% glutaraldehyde mixture buffered with 0.1M phosphate (pH 7.4) for 2 hours, post fixed in 1% osmium tetroxide in the same buffer for 1 hour (Palade, 1952), dehydrated in graded ethanol, and embedded in Epon 812 (Luft, 1961). Ultrasections were made by LKB III ultramicrotome, doubly stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined under JEM-100B type electron microscope.

RESULTS AND DISCUSSION

The nucleus of proximal convoluted tubule cell in control kidney was found at the base of the cell (Fig. 1). Brush border was composed of many regularly and closely packed microvilli. Mitochodria were scattered between basement membrane with uniform thickness and brush border. And they were enclosed mostly by basal infolding located just over the basement membrane. A small number of lysosomes were observed in the cytoplasm (Fig. 1).

While, the nucleus of distal tubule cell was closer to the apical surface than the basal surface, mitochondria between basement membrane with uniform thickness and nucleus were enclosed mostly by basal infolding (Fig. 2).

Basal infoldings are commonly found in cells engaged in active transport of body fluid and ions (Lentz, 1971). On the basis of this fact, mitochondria of proximal and distal tubule cells would be closely associated with the basal infoldings to supply the energy for active transport.

In group I, proximal tubule cell showed short and irregularly arranged microvilli compared with the control, which seems to be due to toxicity of methylmercury chloride (Figs. 3, 4). This is in good agreement with the finding that microvilli were degenerated and separated when rat was treated with chronic mercury chloride (Ganot *et al.*, 1974) and quail was treated with cadmium (Richardson and Fox, 1974). The small vacuoles were near brush border and many large vacuoles containing dense body were found at the middle part of cytoplasm. Mitochondria were considerably swollen and basement membrane was partly thickened (Fig. 4). Numerous lysosomes with low electron density appeared and these lysosomes might discharge methylmercury chloride absorbed in tissues to outside. This assumption could be supported by the report that lysosomes play a role in the digestion of foreign materials in cells (Porter and Bonneville, 1973).

In the distal tubule cell of group I, a small number of little and round lipid droplets were found (Fig. 5). This appearance is considered to be due to the toxicological effect of methylmercury chloride on lipid metabolism. A large number of ribosomes were observed throughout the cytoplasm comparison to the control and this increase might be caused by the separation of ribosomes resulted from the decomposition of rough endoplasmic reticulum due to toxicity of methylmercury chloride. Mitochondria were largely swollen and basal infoldings were slightly developed (Fig. 6). Basement membrane was partially thickened. Necrotic cells with nucleus atrophied and part of cytoplasm were broken away to the inside of lumen (Fig. 5). This implies that distal tubule cells might be damaged by the methylmercury chloride to a great extent.

The thickening of basement membranes in proximal and distal tubules generally occurs in the state of various renal diseases. This thickening is probably affected by the change in permeability due to a failure in reabsorption of convoluted tubule during methylmercury

chloride poisoning induced in the kidney. This assumption is supported by the report of Klaasen *et al.* (1980) that methyl mercury reacts with sulfhydryl groups of membrane proteins and induces morphological alterations such as tubular obstruction, increased back diffusion of tubular filtrate, and inhibition of tubular reabsorption.

In group II, proximal tubule cell showed the decrease of vacuoles in size and number compared with group I (Fig. 7). Mitochondria were slightly swollen and numerous lysosomes were observed.

In the distal tubule cell of group II, cell surface appeared to be more regular than that of group I (Fig. 8). Swelling of mitochondria and thickening of basement membrane were decreased, and nearly recovered to the level of control.

From the above results, the most common prominent change of groups I and II in renal tubule cells was the swelling of mitochondria. This phenomenon would seem due to membrane alterations resulted from the combination of methyl mercury and sulfhydryl groups of mitochondrial membrane. Shimamura (1972) also reported that renal medulla cells showed a variable degrees of swelling of mitochondria after the injection of 2-bromoethylamine hydrobromide in rats. In view of the fact that a major function of mitochondria is generation of energy for synthesis of metabolic materials (Lentz, 1971), it is suggested that the alteration of mitochondria would cause the abnormal renal function.

SUMMARY

The antitoxic effect of red ginseng extract on toxicity of methylmercury chloride to fine structure of mouse kidney was studied by electron microscopy.

The renal proximal tubule cells of the group treated with methylmercury chloride showed considerable swelling of mitochondria and appearance of small or large vacuoles. In the distal tubule cells, remarkable swelling of mitochondria, thickening of basement membrane, increase of ribosomes, and small lipid droplets appeared.

The renal proximal tubule cells of the group treated with methylmercury chloride-red ginseng extract showed slight mitochondrial swelling and apparent reduction of vacuoles in size and number in comparison to the methylmercury chloride treatment group. While, slight swelling of mitochondria and decrease in thickening of basement membrane were occurred in the distal tubule cells, which is similar to the control.

REFERENCES

Arena, J.M., 1986. Poisoning: Toxicology, symptoms, treatments. 5th ed. Thomas Publisher, Illinois, U.S.A. pp. 206-209.

Bae, H.W., 1978. Korean ginseng, 2nd ed. Korea Ginseng Research Institute Publication, pp. 141-151.

Brekhman, I.I. and I.V. Dardymov, 1969. New substance of plant origin which increase nonspecific

resistance. *Ann. Rev. Pharmacol.* **9**:419-430.

Donaldson, M.L. and C.J. Gubler, 1978. Biochemical effects of mercury poisoning in rats. *Am. J. Clin. Nutr.* **31**:859-864.

Fang, S.C. and E. Fallin, 1976. The binding of various mercurial compounds to serum proteins. *Bull. Environ. Contam. toxicol.* **15**:110-117.

Ganote, C.E., K.A. Reimer and R.C. Jennings, 1974. Acute mercuric chloride nephrotoxicity: An electron microscopic and metabolic study. *Lab. Invest.* **31**:633-647.

Guthrie, F.E. and J.J. Perry, 1980. Environmental toxicology. Elsevier North Holland, Inc. New York, pp. 304-336.

Han, B.H., 1974. Metabolism of triterpene glycosides of Korean ginseng(I). *Korean J. Ginseng Sci.* **2**:17-19.

Hodgson, E. and F.E. Guthrie, 1982. Biochemical toxicology. Elsevier Science Publishing Co., New York, pp. 331-336.

Hong, S.A., J.K. Lim and C.W. Park, 1979. Pharmacological action of ginseng. *Korean J. Ginseng Sci.* **3**:66-93.

Klaassen, C.D., J. Doull and M.O. Amdur, 1980. Casarett and Doull's toxicology: The basic science of poisons. 2nd ed. Macmillan Publishing Co., New York, pp. 421-428.

Lentz, T.L., 1971. Cell fine structure: An atlas of drawings of whole cell structure. Saunders Co., Philadelphia, pp. 3-10.

Luft, J.H., 1961. Improvements in epoxy resin embedding method. *J. Biophys. Biochem.* Cytol. **9**:409-414.

Palade, G.E., 1952. A study of fixation for electron microscopy. *J. Exp. Med.* **95**:285.

Porter, K.R. and M.A. Bonneville, 1973. Fine structure of cell and tissues. Kimpton Publisher, London, pp. 68-70.

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**:208-212.

Richardson, M.E. and M.R.S. Fox, 1974. Dietary cadmium and enteropathy in the Japanese quail: Historical and ultrastructural studies. *Lab. Invest.* **31**:722-731.

Shimamura, T., 1972. Drug induced renal medullary necrosis: I. Structural alteration of renal medulla administration of 2-bromoethylamine hydrobormide. *Arch Path.* **94**:406-410.

Valek, A., 1965. Acute renal insufficiency in toxication with mercury compounds. I. Aetiology, clinical picture, renal function. *Acta Med. Scand.* **177**:63-67.

Ware, R.A., L.W. Chang and P.M. Burkholder, 1974. Ultrastructural evidence for foetal liver injury induced by in utero exposure to small doses of methylmercury. *Nature* **251**:236-237.

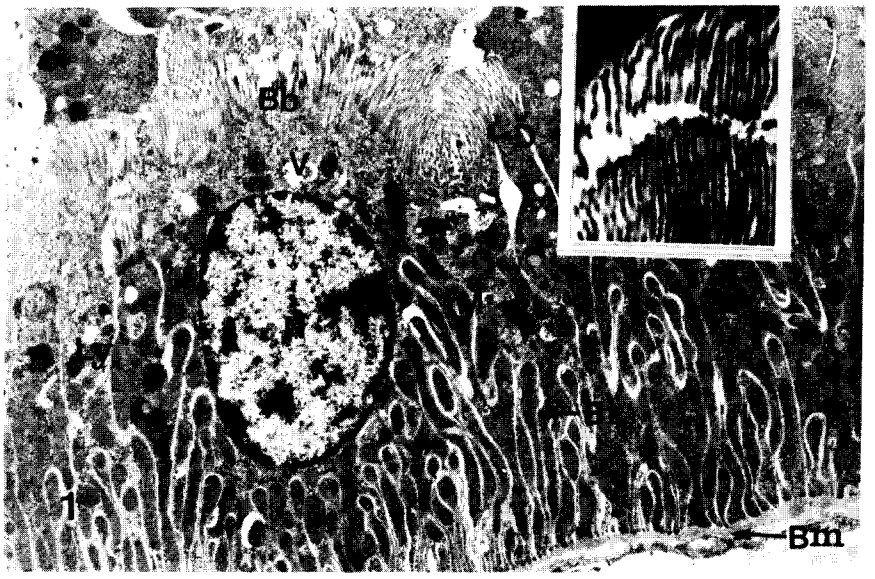

Fig. 1. Renal proximal tubule cell of the control mouse shows the closely and regularly packed microvilli (Mv) of brush border (Bb). The nucleus (N) is at the base of the cell and basement membrane (Bm) has uniform thickness. There are mitochondria (M), some lysosomes (Ly), and vacuoles (V). Arrowhead indicates desmosome (D) and short arrow indicates basal infolding (Bi). ×65,000. (Inset: ×12,500).

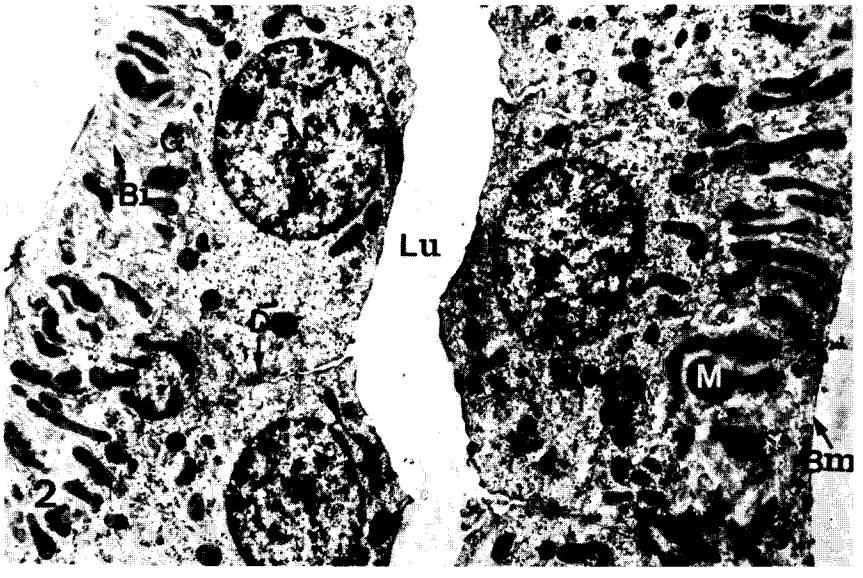

Fig. 2. Distal tubule cell of the control shows basement membrane (Bm) with uniform thickness and normal mitochondria (M). Nucleus (N) is closer to the apical surface. D, Desmosome; Lu, Lumen; G, Golgi body. ×6,000.

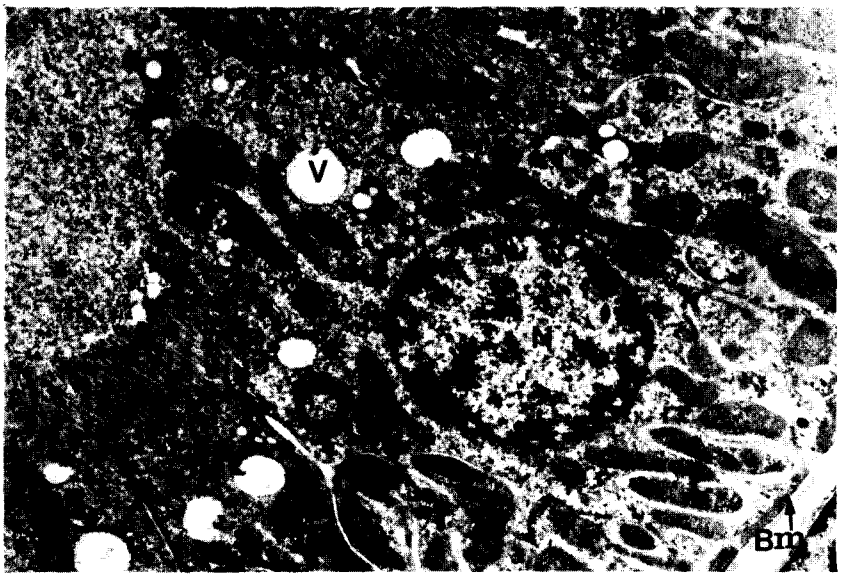

Fig. 3. Proximal tubule cell of the mouse treated with methylmercury chloride. Note the swollen mitochondria (M) with matrix rarefaction. Microvilli (Mv) of brush border (Bb) show somewhat irregular arrangement.

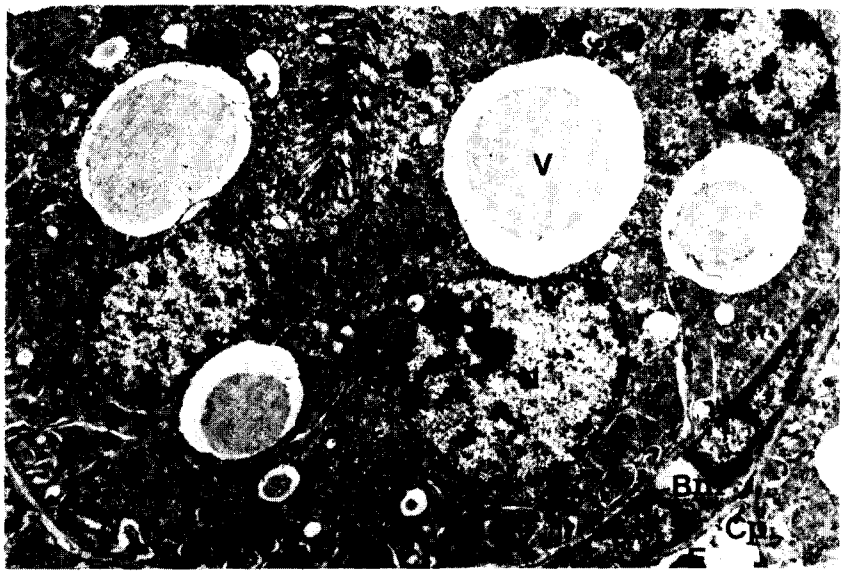

Fig. 4. Proximal tubule cell of the mouse treated with methylmercury chloride. Large vacuoles (V) with dense body are around middle part of cytoplasm. Basement membrane (Bm) is thickened partially. ×6,000.

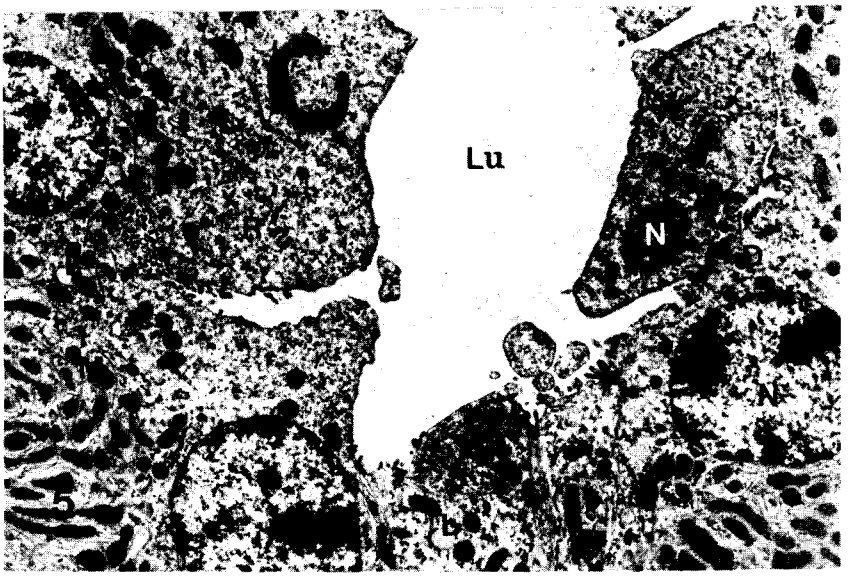

Fig. 5. Distal tubule cell of the mouse treated with methylmercury chloride. There are swollen mitochondria (M) and many ribosomes (R). Basal infolding (Bi) is developed slightly. Necrosis cell (*) and small lipid droplets (L) are observed. ×6,000.

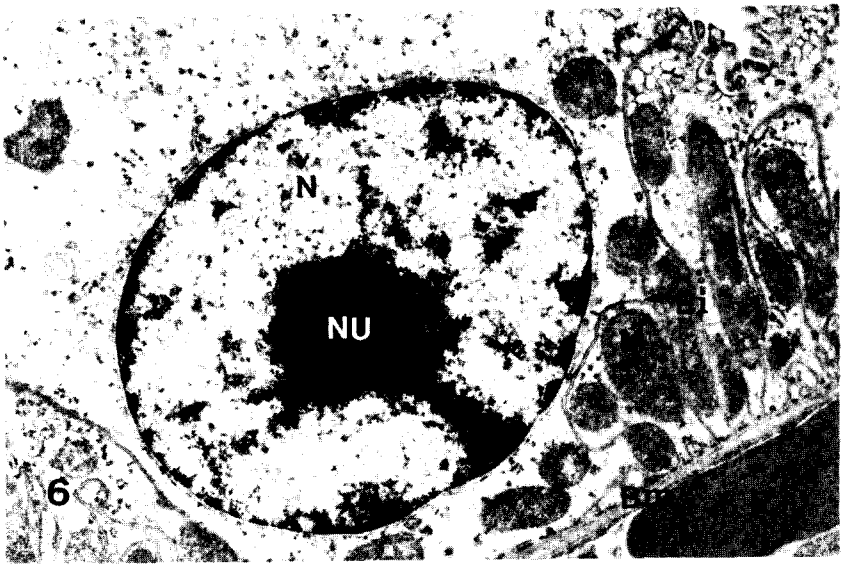

Fig. 6. Distal tubule cell of the mouse treated with methylmercury chloride. Basement membrane (Bm) is thickened. D, Desmosome. ×14,000.

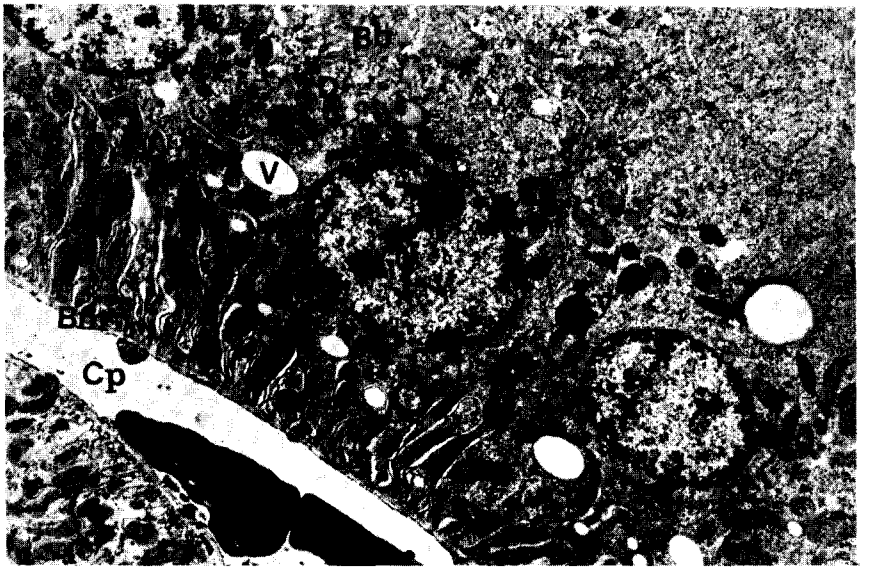

Fig. 7. Proximal tubule cell of the mouse treated with methylmercury chloride-red ginseng extract. It shows many lysosomes (Ly) and some swollen mitochondria (M). V, Vacuole; Bb, Brush border; Bm, Basement membrane; N, Nucleus; Cp,Capillary; E, Erythrocyte; D, Desmosome. ×6,000.

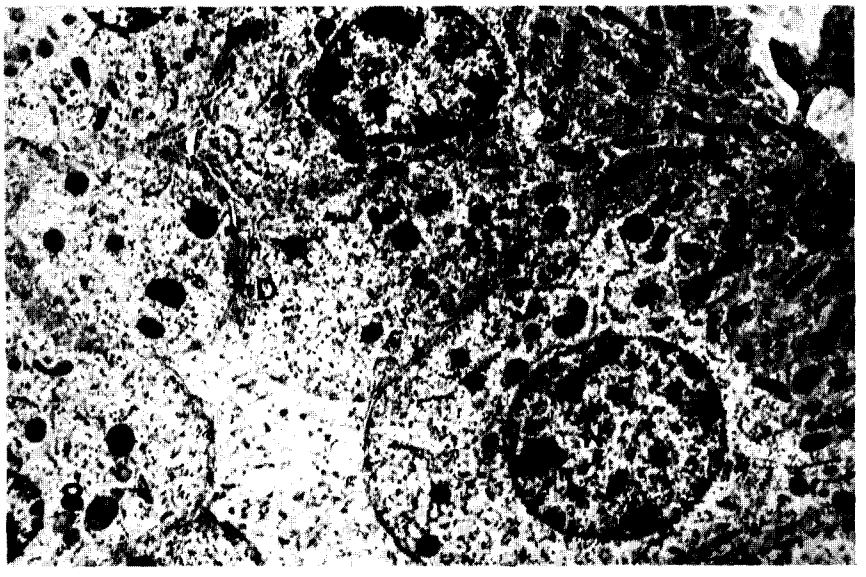

Fig. 8. Distal tubule cell of the mouse treated with methylmercury chloride-red ginseng extract. Nucleus (N), mitochondria (M), and cell membrane surface are similar to those of the control. ×9,000.