Turk J Vet Anim Sci
26 (2002) 1161-1169

Research Article

Effects of Hexachlorobenzene on Rat Renal Cortex: An Ultrastructural Study

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Received: 16.11.2001

Abstract: Male Wistar albino rats, bred and fed under standard laboratory conditions, were administered for 20 days two dosages [Group I: 750 mg/kg body weight (n = 15) and Group II: 1500 mg/kg body weight (n = 15)] of oral hexachlorobenzene resolved in corn oil. Control group animals (n = 15) received only corn oil for 20 days. Twenty-four hours after the administration of the last dose, renal tissue samples, obtained by laparotomy, were fixed in 3% glutaraldehyde in phosphate buffer and 1% aqueous osmium tetroxide. Following the routine tissue processing steps, samples were blocked in Araldyte CY 212. Ultrathin sections taken by LKB V ultratome were stained with uranyl acetate-lead citrate and evaluated under a JEOL 100C transmission electron microscope.

In experimental group I, glomerular features were dilated and hyperaemic capillaries, discontinuous endothelial fenestrae, ondulated and thickened basement membrane in some areas and discontinuous podocyte pedicels were observed. The proximal tubular features had irregular intracytoplasmic foldings and myelin figures in basal and apical cellular regions, whereas distal tubular cells had large, pleomorphic and electron dense mitochondria, microvillous loss and an apical cytoplasm projecting into the tubular lumen.

In experimental group II, renal cortical features revealed irregularly arranged glomerular capillaries which had an ondulated and thickened basement membrane, degenerative endothelial cell fenestrae and irregularities in podocyte pedicels. The proximal tubular cells of the same group contained different size vacuoles with myelin figure-like structures, and giant vacuoles were also present within these cells. Microvilli of the distal tubular cells projecting into the lumen were absent in some regions. There was increased vascularization in the interstitial region and these dilated blood vessels seemed to invade almost the whole intertubular area.

In conclusion, hexachlorobenzene administration might have adverse effects on renal tissue and these effects become worse with increased dosages of the agent.

Key Words: hexachlorobenzene, renal cortex, ultrastructure

Hekzaklorobenzenin Sıçan Böbrek Korteksi Üzerine Etkileri: Ultrastrukturel Bir Çalışma

Özet: Standart laboratuar koşullarında beslenen ve yetiştirilen Wistar albino erkek sıçanlara mısır yağı içinde çözünen [Deney grubu I: 750 mg/kg va (n = 15) ve Deney grubu II: 1500 mg/kg va (n = 15)] hekzaklorobenzenin 2 farklı dozu 20 gün süre ile oral olarak verilmiştir. Kontrol grubunu oluşturan hayvanlara (n = 15) 20 gün süre ile sadece mısır yağı verilmiştir. Son doz uygulanmasından 24 saat sonra sakrifiye edilen sıçanlardan laparotomi ile alınan böbrek dokusu örnekleri gluteraldehit ve osmium tetroksit ile tespit edilmiştir. Rutin protokolu takiben örnekler Araldit CY 212 içinde bloklanmıştır. LKB V ultratomu ile alınan kesitler uranil asetat-kurşun sitrat kontrast boyamasını takiben Jeol 100 C TEM de değerlendirilmiştir.

I. grupta, glomerular kapillerlerin dilate ve hiperemik olduğu, endotel fenestratalarının iyi takip edilemediği, glomerular bazal membranın bazı alanlarda kalınlaşıp ondulasyonlar yaptığı, podosit pedisellerinin düzensizleştiği saptandı. Proksimal tubulus hücrelerinde bazal katlantılarda düzensizlik, bazal ve apikal bölgelerinde lokalize olmuş miyelin figürler izlendi. Distal tubulus hücrelerinde mitokondrinin iri, pleomorfik ve elektron-dens olduğu, mikrovilluslarının silindiği, apikal sitoplazmanın yüzeye doğru çıkıntı yaptığı saptandı.

II.grupta, glomerular kapillerlerde düzensizlik, bazal membranlarda ondulasyon ve kalınlaşma, endotel hücre fenestratalarında dejenerasyon, podosit pedisellerinde düzensizlik izlenmiştir. Proksimal tubulus hücrelerinde, içerisinde miyelin figür benzeri yapıların bulunduğu irili ufaklı vakuoller yanısıra dev vakuollere de rastlanmıştır. Lümene doğru çıkıntı yapan distal tubulus hücrelerinde yer yer mikrovillusların silindiği saptanmıştır. İnterstisyel alanda vaskülarizasyonda artış olduğu ve genişlemiş kan damarlarının intertubular alanın tamamını işgal ettikleri görülmüştür.

Sonuç olarak, hekzaklorobenzenin böbrek dokusu üzerine olumsuz etkileri olduğu ve bu etkilerin doza bağlı olarak arttığı ileri sürülebilir.

Anahtar Sözcükler: Hekzaklorobenzen, böbrek korteksi, ince yapı

Introduction

Hexachlorobenzene (HCB), an environmental contaminant, is an aromatic hydrocarbon. It is found in seaports and shores, and also in whale and human serum in detectable amounts (1-3). HCB is used as a pesticide and fungicide in agriculture (4,5), in the aluminium industry (6,7) and in electrochemical factories (8,9). The effects of HCB on tissues and blood parameters were studied in different species and were found to be immunotoxic (10), carcinogenic, hepatotoxic-hepatocarcinogenic (8,11-12), toxic in the reproductive tract (13), porphyrogenic (6,14-16), and nephrotoxic (4,17-19), and it also had toxic effects on skin, lung and brown adipose tissue (20-22). The best known effect is its hepatic porphyria, which is used in studies as an experimental porphyric agent. Therefore most of the effects of HCB have been studied. Since its effects on kidney morphology have rarely been investigated, the present study was designed to evaluate the effects of two different dosages of HCB on renal cortical ultrastructure.

Materials and Methods

Wistar albino male rats with the mean body weight of 245 ± 5 g, bred and fed in standard laboratory conditions, were obtained from The Experimental Animal Laboratory of Cumhuriyet University. Experimental group animals were administered orally for 20 days two dosages of HCB dissolved in corn oil, 750 mg/kg b.w. (n = 15) and 1500 mg/kg b.w. (n = 15), respectively. Control group animals (n = 15) received only corn oil for 20 days. Twenty-four hours after the last dose, all animals were killed and renal tissues were obtained by laparotomy. Tissues were double fixed in 3% glutaraldehyde and 1% aqueous osmium tetroxide. Tissue samples were blocked in Araldyte CY 212 following standard tissue processing steps. Semithin and ultrathin sections were cut using an LKB-V ultratome, stained in uranyl acetate-lead citrate and evaluated under a JEOL 100C electron microscope.

Results

Control Group

Control group rats, administered corn oil only for 20 days, had glomeruli with regular basement membranes and endothelial cell fenestrae. In addition, podocyte pedicels were regular in appearance (Figs. 1, 2). Proximal tubular cells of this group were on a homogeneous basement membrane, and they had regular abundant intracytoplasmic foldings and electron dense mitochondria among them. Nuclei were euchromatic and had distinct nucleoli. Regular, abundant and high microvilli were the distinctive feature of the proximal tubular cells. Distal tubular cells were located on a thin, homogeneous basement membrane, and they had euchromatic nuclei, abundant mitochondria and rough endoplasmic reticulum in the perinuclear region (Fig. 3).

Experimental Group I

This group of animals received 750 mg/kg b.w. HCB and the most distinctive features of these animals were

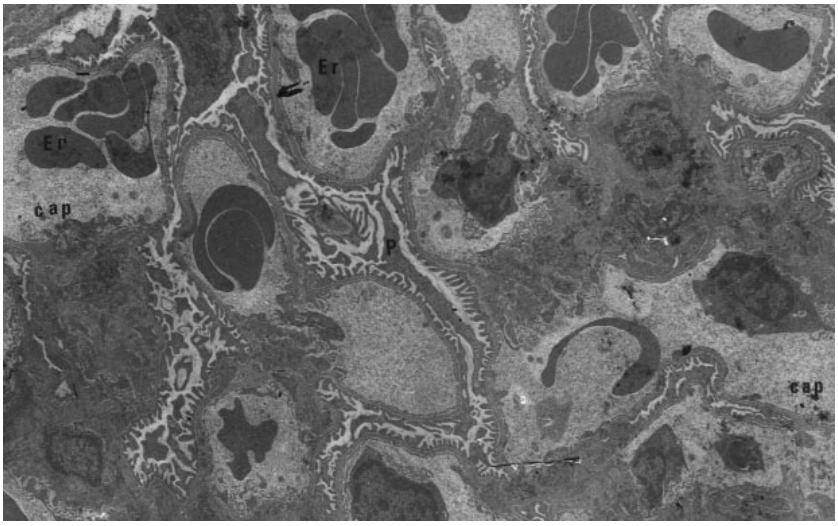

Figure 1. Control group's glomerular structure. Glomerular capillary (cap), regular basement membrane (→), endothelial fenestrae (⇒), erithrocytes (Er), podocytes (P). X 2000.

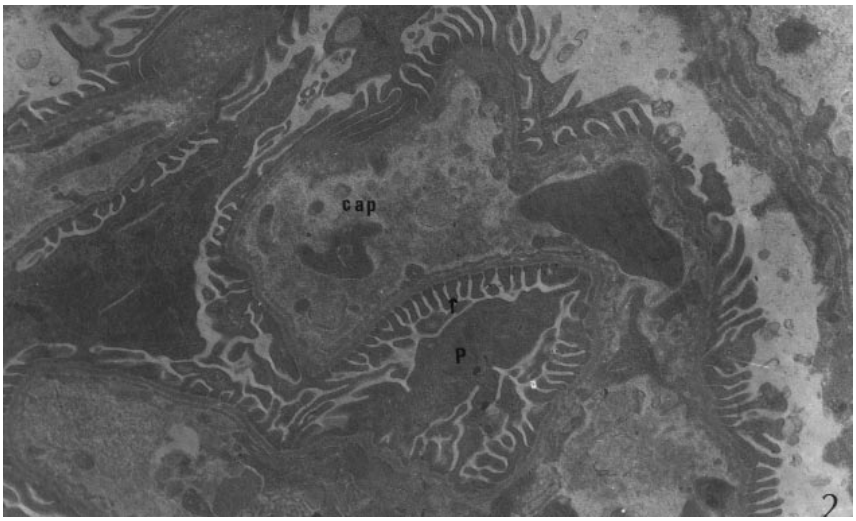

Figure 2. Glomerular capillary (cap) of the control group with higher magnification. Podocytes (P) with regularly arranged pedicels (→) and Bowman's space (Bs). X 3300.

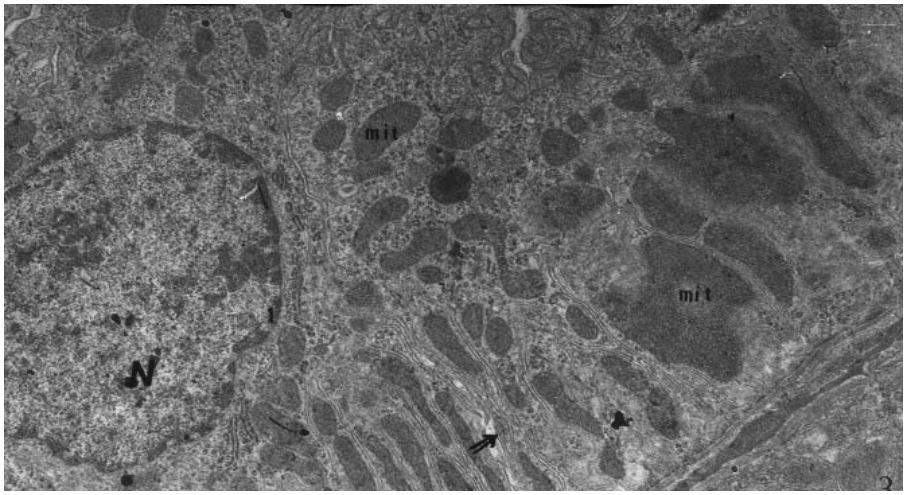

Figure 3. The control group's distal tubular cell with euchromatic nucleus (N), mitochondria (mit), rough endoplasmic reticulum (RER) and intracytoplasmic foldings (⇒). X 3300.

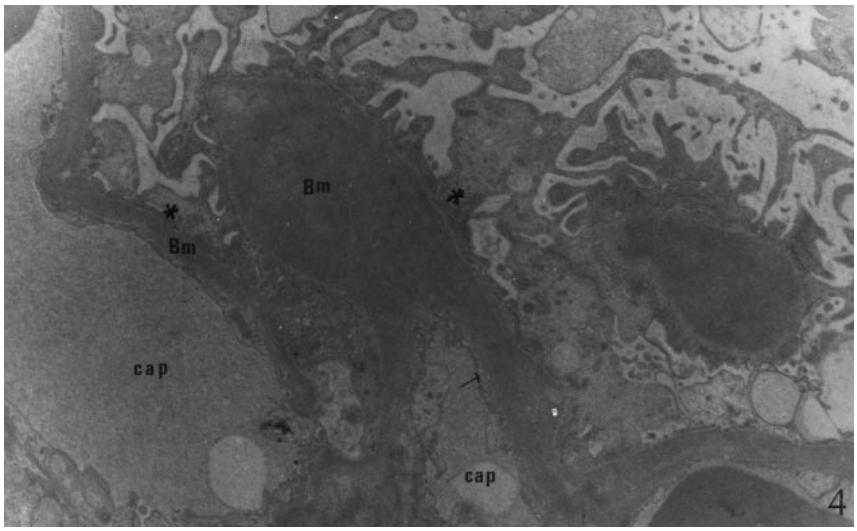

Figure 4. A dilated glomerular capillary (cap) belonging to experimental group I (750 mg/kg b.w. HCB treatment) contains a thickened basement membrane (Bm), irregular endothelial fenestrae (→) and podocyte pedicels (*). X 5000.

hyperaemic and dilated glomerular capillaries along with irregular endothelial fenestrae, thickened and ondulated basement membrane, irregular podocyte pedicels and slit membrane (Fig. 4). Myelin figures both in the apical and basal regions of the cytoplasm and irregularities and dilatations in the intracytoplasmic foldings of the proximal tubular cells were evident (Fig. 5).

Distal tubular cells had large, pleomorphic and electron dense mitochondria; they also lost their microvilli and apical cytoplasmic protrusions were evident (Fig. 5). Lipid droplets were present in the apical cytoplasm of distal tubular cells, whereas they had euchromatic nuclei (Fig. 5). An abundant capillary network was present in the intertubular region of the renal cortex (Fig. 5).

Experimental Group II

These animals, which received 1500 mg/kg b.w. HCB, had glomerular features including irregularly arranged, ondulated and thickened basement membrane, degenerated endothelial cell fenestrae, exfoliation of podocyte pedicels, degeneration of slit membrane and formation of a fibrillar-like cytoplasm in podocytes (Figs. 6, 7). Bowman's spaces seemed to be dilated in some regions (Figs. 6, 7). Proximal tubular cells in this group were degenerated distinctively. In these cells, myelin figure-like structures in vacuoles along with very large vacuoles were also evident. Dilatations in the intracytoplasmic basement membrane foldings and intactly preserved microvilli were observed (Fig. 8). Distal tubuli were dilated. They had cytoplasmic protrusions along with microvillous loss and abundant vacuoles in their cytoplasm (Figs. 9, 10). Interstitial vascularization seemed to be increased and filled almost the entire intertubular region. Those blood vessels were dilated similar to those seen in the glomerular capillaries (Figs. 9, 10).

Discussion

HCB is a well known hazardous, environmental chemical contaminant. Its hazardous effects have been described in agriculture, in which it is used as pesticide and fungicide (3-5), in the aluminium industry (6-7), in the electrochemical industry (8-9) and so on. Kessabi et al. (4) have shown that HCB and lindane were dominant contaminants in eggs, liver and kidneys where lindane was present in 86% and HCB was in 7.5% of all samples. While there are numerous studies on its toxic and carcinogenic effects on several tissues and organs, there have been few reports on the effects of HCB on the renal cortex (4,16,19). Therefore the present study was designed to determine the potential effects of HCB on the rat renal cortex at the ultrastructural level.

The half-life of HCB was found to be 24 days in rats and 32 days in rabbits, whereas it was 8 days and 12 days respectively when animals received combined HCB and hexadecane treatment (23). Following the 14-CHCB injection in rats, HCB became spread out in the body within 2 hours, whereas peak values were determined in liver and brown adipose tissue after 4 hours and in abdominal and subcutenous adipose tissue after 24 hours (24). The highest levels of HCB were found in adipose tissues, bone marrow, skin, Harderian gland, nasal mucosa and intestinal tract (24).

Guliaeva et al. (25) suggested that HCB has little effect on lung and kidney monooxygenases; however, araklor and only HCB induced CYP 1A1 in the kidney.

The effects of several organochlorine (OC) contaminants including HCB have been studied in polar bears and a significant negative OC correlation with IgG levels in blood plasma was found indicating their immunotoxic effect (10). These contaminants threaten not only the adults but also their children via breast milk feeding. Lovelady et al. (26) found HCB and other OCs in breast milk; however, they found no significant relationship between changes in body composition and changes in milk contaminant concentrations in women. However, these contaminants are likely to have adverse effects on the organ systems.

A number of investigators have shown the toxic, carcinogenic and porphyrogenic effects of HCB on several tissues and organs. The hepatocarcinogenic effect of HCB has been reported in rat liver via the formation of glutathione S-transferase (GSTP) positive preneoplastic foci formation (27). An adverse effect of HCB on the female reproductive tract has been suggested in rats by Alvarez et al. (13). They found irregular unoestrus cycle characterized mainly as prolonged periods of oestrus with a reduced number of ova recovered along with significantly decreased uterine nuclear oestrogen receptor levels, degenerative changes in ovarian follicles and germinal epithelium and increased number of atresic follicles (13). In addition, HCB causes an increase in spleen weight, and inflammatory changes in the lung, skin

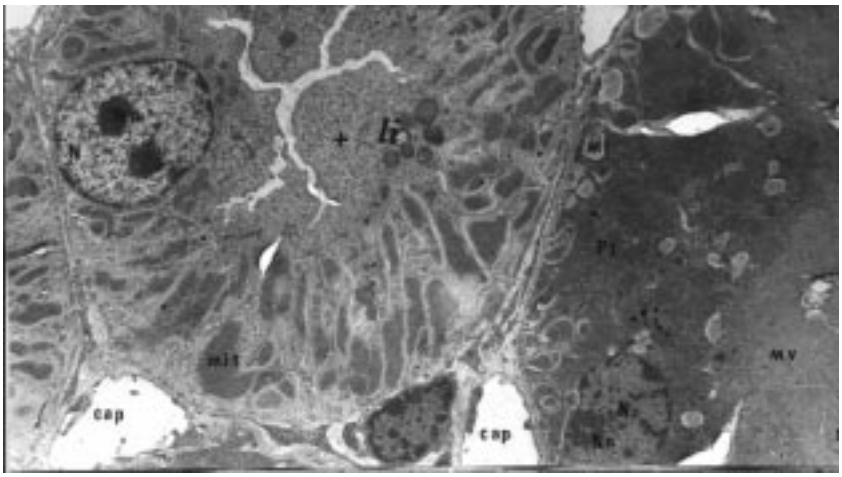

Figure 5. Demonstrates the proximal and distal tubular structures of experimental group I. Proximal tubulus (Pt) has dilated intracytoplasmic foldings (→), myelin figures (mf), microvilli (mv), euchromatic nuclei (N) and nucleoli (Nn). Bleoformation (+), lipid droplets (li), and electrondense pleomorphic mitochondria (mit) are distinct features of distal tubular cells. A wide capillary network (cap) is also present in the intertubular region. X 2000.

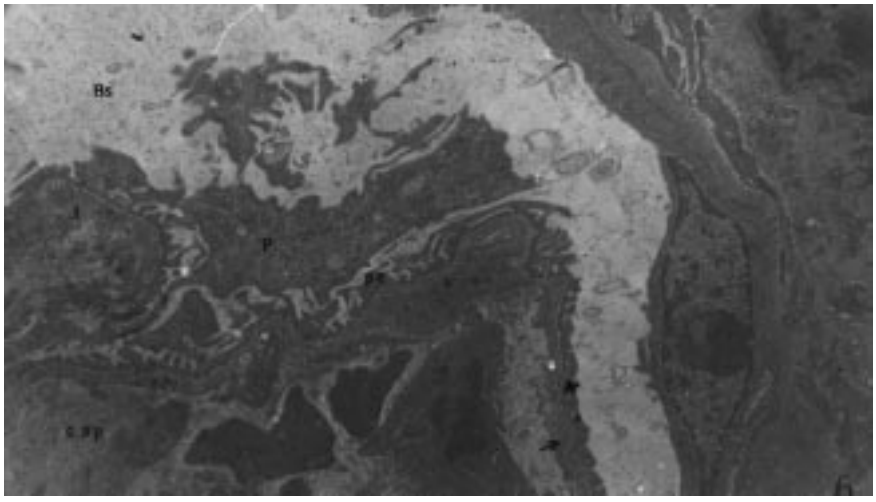

Figure 6. Glomerular structure of experimental group II consists of dilated capillaries (cap) along with their irregular endothelial fenestrae (→), podocyte (P) with its irregular pedicels (pe), detoriorated slit membrane (*) and extended Bowman's space (Bs). X 3300.

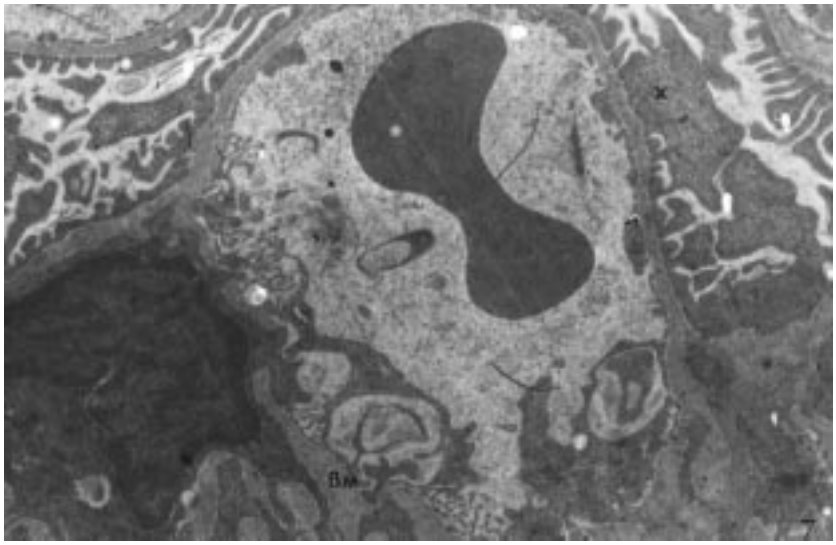

Figure 7. Glomerular features of experimental group II contains irregular endothelial fenestrations (→), thickened basement membrane (Bm) and detoriorated slit membrane (*). X 10,000.

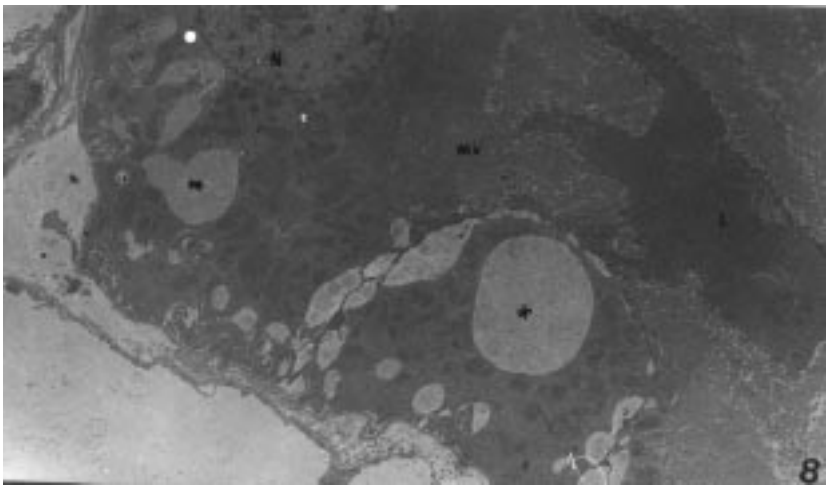

Figure 8. Shows the proximal tubular cell features of experimental group II. There are myelin figure-like structures in vacuoles (*), dilatations in basement membrane foldings (→), euchromatic nucleus (N) with nucleoli and regularly arranged microvilli (mv) in the luminal (L) side of cells. X 2600.

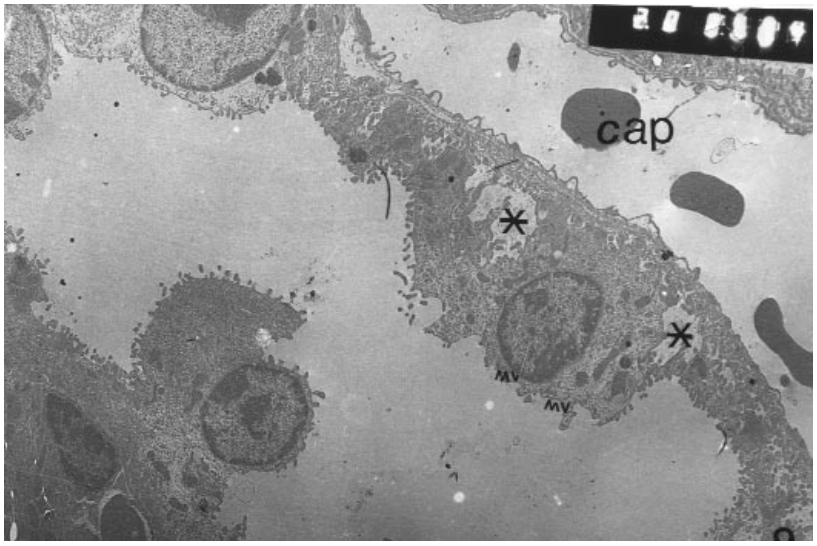

Figure 9. Dilated distal tubular morphology in experimental group II reveals distinctive microvillous (mv) loss, cytoplasmic loss and numerous vacuoles (*). There is also a dilated capillary (cap) in the interstitial region. X 2000.

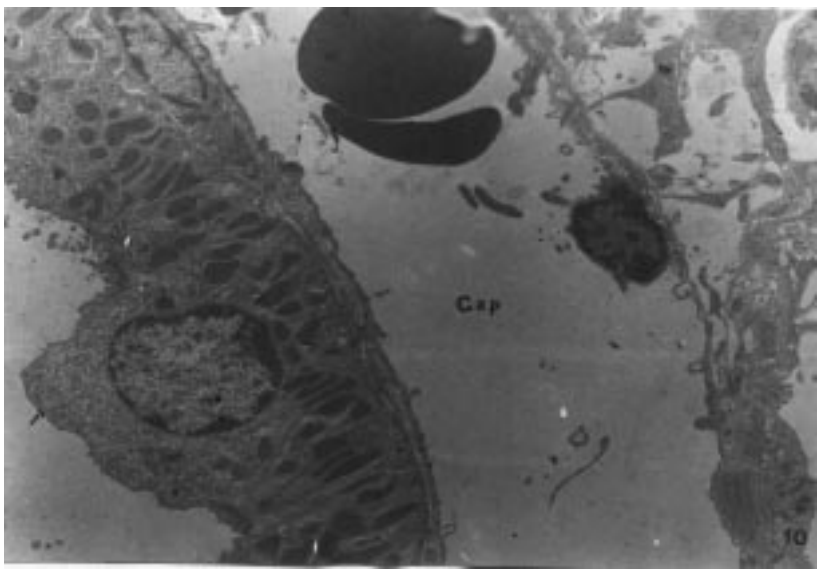

Figure 10. In experimental group II distal tubular cells have microvillous loss (→) and a dilated interstitial capillary (cap). X 2600.

and red pulp of the spleen (22). These inflammatory changes might be related to the immunotoxic effects of HCB.

The effects of HCB on different compartments of the kidney have been evaluated by Fernandez-Tome et al. (16). To do this, they measured conjugated diene and malondialdehyde levels as lipid peroxidation parameters and porphyrin accumulation as uroporphyrinogen decarboxylase activity in the renal cortex, medulla and papilla. Their results demonstrated a direct correlation between the oxidative environment and the effect elicited by the drug on the heme metabolism in the renal cortex, whereas HCB showed no porphyrogenic effect in the papilla and medulla where the antioxidant systems were higher.

In a histological study of several tissues based on the effects of the porphyrogenic and carcinogenic ability of HCB, several tissues were examined such as the liver, thyroid, spleen and kidney of male and female golden hamsters by Rios de Molina et al. (5). At the end of ten months of HCB feeding, while they found the thyroid gland and kidney unaltered, the liver and spleen exhibited morphological alterations such as hypotrophic red pulp less developed with respect to the Malpighian corpuscle and many macrophages with iron deposits in the spleen and enlarged hepatocytes, high content of iron deposits, small lipid droplets, polimorphic nuclei and microsteatosis in the liver (5). They concluded that HCB is able to cause precancerous pathology and induce porphyria. Although they found an unaltered kidney histology following HCB treatment, the results of the present study revealed distinctive renal cortical ultrastructural changes following HCB administration; 750 mg/kg b.w. HCB treated rat renal cortex demonstrated glomeruli with a thickened basement membrane, and hyperaemic and dilated capillaries, whereas proximal tubular cells had myelin figures in their cytoplasm and increased intracytoplasmic foldings. On the other hand, distal tubular cells had large, electron-dense mitochondria along with a significant microvillous loss. Cortical alterations were more distinctive when the animals were treated with an increased amount (1500 mg/kg b.w.) of HCB. These features were the exfoliation podocyte pedicels, degeneration of slit membrane and fibrillar-like cytoplasm in podocytes. Proximal tubular cells were distinctively degenerated whereas distal tubular cells had apical cytoplasmic protrusions with microvillous loss and numerous vacuole-like structures in their cytoplasm. In addition, an increased vascularization was observed in the interstitial region possibly indicating a precancerous condition in renal cortex following a high dose administration of HCB.

Lecavalier et al. (28) suggested increased relative liver weights accompanied by mild moderate morphological changes in HCB treated rats. These histological changes were more severe in HCB + mercury treated animals, indicating the combined effects of these chemicals. The oncogenicity of HCB has been indicated and renal adenomas were shown in several laboratory animals (11). Their findings may indicate a change in genetical mechanisms caused by HCB, leading to precancerous and finally cancerous alterations in tissues.

There have been numerous reports on the ultrastructural effects of HCB (29-34). Bourque et al. (29) administered this pollutant orally at dosages of 0.01 and 10 mg of HCB/kg of body weight to monkeys. They suggested abnormal cytoplasmic lipid accumulation, herniation of the ooplasm, and degenerative follicular cells in the ovaries of monkeys given 0.1 to 1.0 mg of HCB. An increased amount (10 mg/kg) of HCB caused mitochondrial degenerations. Babineau et al. (30) suggested that the HCB treated animals contained large number of lysosomes, and numerous vesicles at the surface epithelia of ovaries. On the other hand, MacPhee et al. (31) suggested dilated smooth endoplasmic reticulum and Golgi complexes along with prominent free polysomes in the corpora lutea from Sprague Dawley rats. Mollenhaur et al. (32,33) suggested proliferated SER structures and accumulation on glycogen-like material along with elongated and swollen mitochondria, degenerative lipid vesicles leading to a form of autophagic vacuole or a storage vesicle in rat hepatocytes. Siersema et al. (34) found needle-like structures, and representive uroporphyrin crystals in rat hepatocytes following HCB administration. The results of the present study revealed similar mitochondrial alterations in both experimental groups. In particular, distal tubular cells had large, electron-dense mitochondria.

In conclusion, HCB causes severe alterations in the renal cortical structure and the use of HCB must be limited or strictly controlled in order to protect people from the severe effects of hexachlorobenzene.

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