THE MORPHOLOGIC EFFECTS OF DIELDRIN AND METHYL MERCURIC CHLORIDE ON THE PROXIMAL TUBULES OF RAT KIDNEY

by

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TABLE OF CONTENTS

INTRODUCTION

Technological processes often result in dissemination of potentially toxic substances into the world ecosystem. The subsequent uptake and concentration of these toxic substances by living organisms is cause for concern. Two groups of chemicals, the chlorinated hydrocarbon pesticides and organo-mercury compounds, are examples of environmental pollutants that can be concentrated in biological systems and which are potentially toxic to man.

Chlorinated Hydrocarbon Pesticides

During the past twenty-five years, the chlorinated hydrocarbon pesticides (DDT, toxaphene, aldrin, dieldrin, endrin and chlordane) have played a major role in the control of insect-borne diseases. Eradication of destructive insects by these compounds has increased crop production in many areas of the world. The value of these insecticides rests with their general effectiveness, retention within the environment for long periods of time, and relatively low cost.

Problems with chlorinated hydrocarbon pesticides have occurred because of their extreme stability, high concentrations in the upper levels of the food chain, and deleterious effects on avian species reproductivity. The chlorine-substituted benzene rings of these compounds possess increased resonance properties which make them resistant to degradation except by ultraviolet light (19). This has increased their value as pesticides, but has allowed them to remain in the environment for years.

The hydrophobic nature of chlorinated hydrocarbons has allowed them to pass across biological membranes and accumulate in lipid-containing tissues. Concentrations of these insecticides are increased at each successive trophic level through the process of "biological magnification". Although chlorinated hydrocarbon concentrations are only a fraction of a part per million (ppm) in rivers, lakes and oceans, they may be increased to several parts per million in aquatic and marine microorganisms (43, 79). Fish and predatory bird species may have tissue pesticide concentrations in the ranges of ten to thousands parts per million (43). The presence of chlorinated hydrocarbons in predatory birds has interfered with their reproductive processes (24, 65, 74, 75, 79), and has brought the eagle, peregrine falcon and brown pelican to near extinction.

Chlorinated hydrocarbon pesticides decrease hormonal levels in female birds by induction of liver detoxification enzyme systems which also function to hydroxylate steroid hormones for excretion (65, 74, 75). High hormonal levels are necessary for calcium storage in bones and subsequent deposition in egg shells. Decreased hormonal levels interfere with this process and result in thin egg shells which are easily broken (24, 74, 75, 79). The ecological impact of decreased predatory bird species cannot as yet be predicted.

The effects of chronic exposure to these pesticides on mammals have not been thoroughly investigated. Numerous studies carried out on persons frequently exposed to chlorinated hydrocarbons, such as sprayers, agricultural workers and those employed in pesticide manufacturing plants, have produced little conclusive data.

Hayes and Curley (42), investigating workers at a pesticide manufacturing plant, found significantly higher levels of dieldrin and aldrin than in the general population. They noted a direct correlation between levels of pesticides in serum, urine and adipose tissue, but observed no correlation between levels of dieldrin and sick leave.

Tocci *et al.* (97) measured biochemical differences between pesticide sprayers and formulators and control groups from the general population. They found decreased serum and red blood cell cholinesterase activities in exposed persons. Conversely, serum glutamic oxaloacetic transaminase activity was increased. Serum creatinine and plasma amino acid levels were also elevated. These investigators concluded "...that heavy exposure to pesticides does cause changes in kidney or liver function and in the concentrations of circulating amino acids in about 30% of the people studied." They also noted that, "In addition, specific renal tubular transport defects have been described in 16% of this population."

Most of the experimental animal research on chlorinated hydrocarbon pesticides has centered on alterations of liver metabolism. Proliferation of smooth endoplasmic reticulum (SER) has been reported by numerous investigators (48, 49, 52, 67, 72, 73) in livers of animals exposed to these pesticides. Hutterer *et al.* (49) noted induction by dieldrin of aniline hydroxylase and cytochrome P-450. They also observed a gradual decrease in oxidative phosphorylation with continued exposure to dieldrin.

Schwark and Ecobichon (84) noted induction of liver carboxy esterase activity with DDT poisoning. Tocci *et al.* (97) reported that DDT caused a decrease in glucose-6-phosphate dehydrogenase levels in rat

liver, while inducing microsomal detoxification enzymes. These authors also noted induction of aldolase and alkaline phosphatase activities by chlorinated hydrocarbon pesticides.

There have been few investigations of the effects of chlorinated hydrocarbon pesticides on the kidney, although moderately high levels of pesticides have been reported in this organ (30, 66, 110). Schwark and Ecobichon (84) reported that, in contrast to liver, esterase activity was not induced by DDT in rat kidney. They concluded, "Furthermore, the kidney, owing to its ability to concentrate drugs for excretion, may suffer certain detrimental effects, leading to an overall depression of cellular activity and protein biosynthesis." Boyd and co-workers (10, 11, 12, 13) described renal congestion and fatty degeneration of kidney tubules in rats fed high levels of chlorinated hydrocarbon pesticides. This is similar to the effect of carbon tetrachloride on these tubules.

Several authors have indicated that chlorinated hydrocarbon pesticides can cause renal damage. Sowell *et al.* (86) cited the work of Zavon (110), which showed that, after prolonged absorption of endrin, brain, liver, kidney and adrenal tissues of rats displayed diffuse degenerative changes. Necrosis of the proximal and distal convoluted kidney tubules was observed. Treon *et al.* (98) described diffuse degenerative changes in the liver and kidneys of a number of laboratory animals poisoned with endrin, including severe necrosis of the convoluted tubules. These investigators also noted "enlargement" of the kidneys in dogs fed endrin at a concentration of 8 ppm for 6 months.

Energy-producing systems which supply active transport processes seem to be affected by these pesticides. Hosein and Proulx (45) found that dieldrin decreased oxygen consumption by rat brain slices and that this effect was due to damage of neuronal mitochondria. Sowell *et al.* (86) stated that the inhibition was due to the action of dieldrin on cytochromes and dehydrogenases.

Colvin and Phillips (18) described endrin inhibition of mitochondrial enzymes from livers and brains of fish. The findings cited support the concept that active transport mechanisms within the kidney may be altered by chlorinated hydrocarbon pesticides.

The above observations, and findings of increased creatinine and amino acids in the blood of persons chronically exposed to chlorinated hydrocarbons, suggest that alteration of renal tubular function may occur in humans due to these pesticides.

Inorganic and Organic Mercury

Mercury byproducts from industrial processes, mercurial fungicides used in agriculture, and erosion of mercury-containing salts from ores are major sources (2, 58) of organic and inorganic mercury in the environment. Inorganic mercury derived from the above sources can be methylated by bacteria (50, 109). Wood *et al.* (109) found that extracts from a mixed culture of *Methanobacterium omelianski* could methylate inorganic mercury to dimethyl mercury. This compound, like the chlorinated hydrocarbon pesticides, is lipophilic and capable of crossing biological membranes. Dimethyl mercury dissociates at acid pH to methyl mercury and methane gas. Methyl mercury, which has an

affinity for protein, is taken up by marine and aquatic microorganisms. It is increased in concentration at each trophic level in a manner similar to the chlorinated hydrocarbon pesticides (2, 28, 29, 39, 58).

Human poisonings have occurred in Minamata and Niigata, Japan, from ingestion of fish containing high levels of organic mercury (28, 29, 58). Poisoned individuals presented with severe neurological disorders resulting from concentration of organic mercury in the central nervous system.

The effects of chronic exposure to low levels of methyl mercury on mammals are not well known. Clinical investigations (51, 96) of persons occupationally exposed to organo-mercury compounds have demonstrated a significantly increased proteinuria.

Experimental studies (1, 63, 94, 100, 101, 102) have shown that mercury derived from organo-mercury compounds is concentrated in the kidneys. Other investigators (17, 70, 95) have reported that organo-mercury compounds are metabolized *in vivo* to inorganic mercury. This conversion could cause renal dysfunction because inorganic mercury is highly toxic to the proximal tubules (20, 37, 44, 81). Histopathologic evaluation of kidney tissue from persons or animals exposed to low levels of methyl mercury for extended periods is presently not available.

The study reported in this thesis was undertaken to evaluate the ultrastructural effects on rat kidney of chronic exposure to low doses of the pesticide dieldrin and methyl mercuric chloride. This investigation is part of a comprehensive program currently underway at the Environmental Health Sciences Center of Oregon State University

to elucidate the biological effects of chronic exposure to numerous environmental toxicants. The findings presented here will ultimately be correlated with blood analyses and tissue residue levels. The latter data are currently being assembled by other investigators at the Environmental Health Sciences Center.

MATERIALS AND METHODS

The animals used in this study were taken from a larger population exposed to environmental toxicants. They were a part of a comprehensive experiment carried out by the staff of the Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon. Kidney tissue for the investigations described here was made available when the animals were killed.

A total of 84 inbred Oregon State University-Wistar rats, consisting of 39 males and 45 females, were housed in sterile chambers (40) for the duration of the experiment. Dieldrin, methyl mercuric chloride (CH_3HgCl) and the combination of dieldrin plus CH_3HgCl were added to the daily diet of the experimental animals. All experimental and control animals received a stock laboratory ration described by Harr *et al.* (41). The following groups of animals were studied for the time periods indicated:

Regimen (Level in Diet)	Number of Rats		Days of Diet	Age at Death (days)
	Male	Female		
Dieldrin (5.0 ppm)	8	8	84	112
	7	6	142	170
CH_3HgCl (2.0 ppm)	3	6	84	112
	3	4	142	170
Dieldrin (5.0 ppm) + CH_3HgCl (2.0 ppm)	2	2	84	112
	3	4	142	170
Control	7	11	84	112
	6	4	142	170

Two groups of control animals were maintained on the standard laboratory diet for 112 and 170 days of age respectively.

The animals were anesthetized with ether at the time of sacrifice and their right kidneys excised. Each kidney was cut transversely into pieces.

Samples from each kidney piece were fixed for light microscopy in 10% formalin. This tissue was dehydrated in a 50% to 100% graded series of alcohols and embedded in paraffin. Five micron thick sections were cut on A. O. Spencer 820 microtomes and stained with hematoxylin and eosin by the staff of the Histopathology Laboratory, Department of Pathology, University of Oregon Medical School.

Adjacent sections from selected mercury-treated female and control female rats killed at 84 days were stained by the PAS technique. These sections were examined and photographed with a Zeiss microscope.

For electron microscopy, tissue blocks about 1 cu mm in volume were cut from samples of both inner and outer cortex of all kidneys. These blocks were immersed in one of three fixatives:

Fixative 1:

Glutaraldehyde 2.5%
Formaldehyde 2.0%
$CaCl_2$. . . . . 250 mg/l
0.13 M cacodylate buffer (pH 7.4)

Fixative 2:

Glutaraldehyde 2.5%
Formaldehyde 2.0%
$CaCl_2$. . . . . 250 mg/l
0.04 M cacodylate buffer (pH 7.4)

Fixative 3:

Glutaraldehyde 2.5%
Formaldehyde 2.0%
$CaCl_2$. . . . . 250 mg/l
0.085 M cacodylate buffer (pH 7.4)

In addition, the kidneys of 4 mercury-treated and 4 control female rats killed at 84 days were perfused via cardiac puncture with a Ringer's-procaine solution (31) and then with fixative 3 in 3% sucrose.

The samples were fixed in the above solutions for 3 hours, then transferred to a solution of 1.5% osmium tetroxide buffered with phosphate (pH 7.4) for 2 hours at room temperature (64). Tissues were then dehydrated in a 50% to 100% graded series of alcohols, passed into propylene oxide and embedded in Araldite according to the method of Luft (59). Thin sections (600-900 Å) were cut on an LKB ultrotome and mounted on 300 mesh uncoated copper grids. The sections were double stained with lead citrate (77) and 3% uranyl acetate and examined with an RCA EMU 3-G or Philips EM 200 electron microscope. In addition, 1 μ thick sections of tissue embedded in Araldite were cut and stained with methylene blue-azure II (78) for light microscopy.

RESULTS

The anatomy, histology and ultrastructure of rat kidney have been described extensively by numerous investigators (8, 26, 61). The histology and ultrastructure of kidneys from control rats in this study did not vary from these descriptions, regardless of animal age. In the experimental animals, morphologic alterations of the nephron were limited to the proximal tubule (pars convoluta and pars recta). None of the other components of the nephron showed morphological changes. Consequently, the proximal tubule is the only segment that is extensively illustrated.

In a limited number of animals (10%) receiving dieldrin or dieldrin plus CH_3HgCl, an acute arteritis was observed which will be discussed separately.

Control Animals

Histologic sections of kidneys from both male and female control rats were indistinguishable from each other and possessed a normal morphology (Figure 1). The convoluted segments (pars convoluta) are visible around the juxtamedullary glomeruli. The straight descending segments of the proximal tubule (pars recta) extend into the outer stripe of the medulla (Figure 2) and are continuous with the descending thin limb of Henle.

Cells of the pars convoluta are characterized by a high profile, brush border, numerous mitochondria, and basal cellular interdigitations (Figure 3). Cytosomes and microbodies also are common.

The pars recta has cells with a lower profile, brush border, fewer mitochondria, and little or no basal interdigitations (Figure 4). Microbodies are frequently observed in this segment. Small aggregates of SER were occasionally observed in the apical cytoplasm of cells from both portions of the proximal tubule. These were seen more frequently in female animals.

Animals Exposed to 5.0 ppm Dieldrin in the Diet

Histologic sections from most animals of both sexes were unremarkable and indistinguishable from controls (Figure 5).

In about 10 percent of the rats exposed to dieldrin or mercury plus dieldrin an infiltrate composed of neutrophils, lymphocytes and eosinophils was noted around medium and large arteries (Figures 6 and 7). Elastin staining of an adjacent section revealed an intact tunica intima in affected arteries.

The ultrastructural effects of dieldrin on either portion of the proximal tubule did not seem to be related to duration of exposure for either sex. Increased amounts of SER, damaged mitochondria and degenerating tubule cells characterized dieldrin-treated animals of both sexes (Figures 8, 9, 10, 11 and 12). Proximal tubule cells from some female rats displayed vacuoles containing flocculent material (Figure 9). Proximal tubule cells from male rats showed a greater increase in SER aggregates than was observed in females (Figure 12). Tubule alterations of animals exposed to dieldrin are summarized in Table 1.

Table 1

THE ULTRASTRUCTURAL EFFECTS OF DIELDRIN AND CH_3HgCl

IN COMPARISON TO CONTROL ANIMALS

Changes in Proximal Tubule Cells	Male		Female	
	Pars Convoluta	Pars Recta	Pars Convoluta	Pars Recta
	Dieldrin			
Degenerating Cells	+	+	+	+
Degenerating Mitochondria	+	±	+	+
Increased Numbers of Cytosegresomes	-	-	-	-
Dense Membranous Cytosomes	-	-	-	-
Increased SER	++	++	+	+
SER-Containing Cytoplasmic Masses	-	-	-	-
Vacuoles	-	-	+	+
	CH_3HgCl			
Degenerating Cells	+	+	+	+
Degenerating Mitochondria	+	+	+	+
Increased Numbers of Cytosegresomes	++	+	-	-
Dense Membranous Cytosomes	-	-	-	+++
Increased SER	±	+	±	±
SER-Containing Cytoplasmic Masses	-	-	-	+++
Vacuoles	-	-	-	±
	Dieldrin + CH_3HgCl			
Degenerating Cells	±	±	±	±
Degenerating Mitochondria	-	-	-	-
Increased Numbers of Cytosegresomes	-	-	-	-
Dense Membranous Cytosomes	-	-	-	+
Increased SER	±	+	±	±
SER-Containing Cytoplasmic Masses	-	-	-	±
Vacuoles	-	-	-	-

Animals Exposed to 2.0 ppm CH_3HgCl in the Diet

The effects of CH_3HgCl on the proximal tubules of exposed animals did not increase with duration of exposure for either sex. Morphologic alterations of proximal tubule cells from female rats was different and more marked than in males (Table 1).

Female Rats.

Histologic sections of kidneys from female animals displayed dilatation of the pars recta segments and the presence of hematoxylin-positive, PAS-negative spherical cytoplasmic masses in the lumens (Figures 13, 14, 15 and 16). Pars convoluta cells were indistinguishable from those of controls (Figure 17). Similar cytoplasmic masses were present in the patent lumens of pars recta segments from perfusion-fixed tubules. These masses were characterized ultrastructurally by the presence of an SER bundle (Figure 18). Occasionally, isolated spherical cytoplasmic masses containing a single bundle of SER were noted in the apical cytoplasm of pars recta cells from affected kidneys (Figure 19). Pars recta cells from these females also exhibited numerous electron-dense membranous cytosomes, myelin-like residual bodies, and degenerating mitochondria (Figures 20 and 21). Large aggregates of SER also were observed in these cells (Figure 21).

Male Rats.

Paraffin-embedded sections of kidneys from males given 2.0 ppm CH_3HgCl in the diet could not be distinguished from controls (Figures 22 and 23). Pars convoluta cells of males in this group displayed

increased numbers of degenerating cells and autophagic cytosegresomes, some containing altered mitochondria (Figures 24 and 25). Microbodies were frequently seen partially encircled by endoplasmic reticulum (Figure 26). Some microbodies were observed to be completely isolated from the cytoplasm in cytosegresomes (Figure 27).

Pars recta tubules from males exposed to methyl mercury showed prominent cytosegresomes and degenerating cells in comparison to controls (Figure 28), and those tubule cells which appeared viable contained more SER than was observed in controls.

A summary of the effects of CH_3HgCl on the proximal tubules of experimental animals is given in Table 1.

Animals Exposed to 5.0 ppm Dieldrin plus 2.0 ppm CH_3HgCl in the Diet

Morphologic alterations observed in kidneys of animals exposed to dieldrin plus CH_3HgCl did not seem to progress with time and were less extensive than in those animals given either compound alone.

Female Rats.

Examination of kidney sections from female rats by light microscopy disclosed moderate dilatation of the pars recta tubules, but decreased numbers of cytoplasmic masses within tubule lumens in comparison to females exposed to CH_3HgCl only (Figures 29, 30, 31 and 32). Pars convoluta cells possessed a relatively normal morphology (Figure 33). Cells of the pars recta had some small aggregates of SER and fewer dense membranous cytosomes than observed in females receiving only CH_3HgCl (Figure 34).

Male Rats.

Histologic sections of kidney from male rats fed dieldrin plus mercury in the diet were unremarkable (Figure 35). Morphology indistinguishable from controls was observed in pars convoluta cells of males on this regimen (Figure 36). Infrequently a degenerating cell was seen in a tubule lumen (Figure 37). Cells of the pars recta segments from these males showed somewhat more SER in comparison to controls, but they were otherwise normal in appearance (Figure 38).

A summary and comparison of the effects of dieldrin plus CH_3HgCl on the proximal tubules in relation to the other regimens is presented in Table 1.

DISCUSSION

Low doses of dieldrin and CH_3HgCl produce different toxic effects in kidneys of rats exposed for long periods of time. The severity of lesions in the proximal tubules seems more dependent upon the sex of animals than on the duration of exposure. Administration of both compounds together was not additive, but appeared to result in less cellular change than when either compound was given alone.

Increased amounts of SER were noted in male and female animals given dieldrin. Several investigators (48, 49) have associated proliferation of SER with induction of microsomal enzyme systems. Biochemical studies (3, 14, 23, 34, 53, 56, 57) have shown dieldrin to be a potent inducer of microsomal enzymes. The proliferation of SER in proximal tubule cells probably represents a cellular attempt to detoxify dieldrin to its hydrophilic major metabolite (85), 6,7-trans-dihydrodihydroxy-aldrin and other metabolites.

Ultrastructural alteration of proximal tubule cells was more prominent in female rats than males. The increased numbers of degenerating mitochondria and large vacuoles containing lipidic flocculent material in proximal tubule cells of some females would suggest that a metabolic alteration was caused directly or indirectly by dieldrin.

The presence of vacuoles and lipid accumulation in renal tubule cells has been reported with a variety of agents that alter mitochondrial function (20, 37, 81, 93). Inhibition of mitochondrial respiration is a possible mechanism by which fatty accumulation may

occur (80). The cyclodiene pesticides (dieldrin, endrin) have been reported to inhibit mitochondrial enzymes (18, 86).

The more marked effects of dieldrin on the proximal tubules of female rats may be explained by sexual differences in detoxification enzyme activities. Numerous investigators (23, 55, 56) have reported that the activity level of liver microsomal enzymes is higher in the adult male rat than in the adult female. Sexual differences have also been reported (55, 82, 83, 103, 104) for a number of renal enzymes. Koerner and Hellman (55) found that the activity of the microsomal enzyme 11β-hydroxysteroid dehydrogenase in kidneys of male Wistar rats was twice that of kidneys from female Wistar rats. In the present study, proximal tubule cells from dieldrin-treated male rats showed a greater increase in SER than those of dieldrin-treated females. This suggests a greater intrinsic responsiveness by males to dieldrin. Lower microsomal metabolic activity is probably a major factor in the susceptibility of female rat kidney to dieldrin toxicity. Since dieldrin would not be detoxified to the same extent it could render a greater effect on mitochondrial and other enzyme systems than in male rat kidney.

The kidneys of female rats were also more severely affected by CH_3HgCl than were those of male rats, particularly in the pars recta segments. Organo-mercury compounds are known to be converted to inorganic mercury in the kidney (17, 70, 95, 107), where they are concentrated to high levels (1, 4, 5, 6, 7, 15, 33, 94, 100, 101, 102). Inorganic mercury has long been known to selectively damage the pars recta of the proximal tubule (20, 37, 44, 81). Cellular necrosis,

swollen mitochondria and proliferation of SER characterize acute inorganic mercury poisoning.

The more prominent effects of CH_3HgCl on female proximal tubule cells may also be attributed to differences in renal detoxification enzymes.

Mercury derived from methyl mercury has been reported to concentrate in the microsomal fraction of rat kidney and liver (68, 69). The SER is a logical site for the conversion of methyl mercury to Hg++ because detoxification enzyme activities are present in microsomes. It seems likely that cleavage of the carbon-mercury bond could release inorganic mercury which might then react with microsomal enzymes as a non-competitive inhibitor. Mercurials are known to strongly inhibit liver enzymes of sterol biosynthesis found in the microsomal fraction (106). The mechanism by which the mercurials cause enzyme inhibition is through combination of mercury with SH groups usually present at the active sites of many enzymes (105).

If mercury inhibits these enzyme systems in the kidney, the selective extrusion of SER bundles by pars recta cells of female animals may represent the removal of non-functional organelles through the process of potocytosis (16). These authors (16) feel that potocytosis "...is most probably an active or passive process which attempts to reestablish metabolic equilibrium within the cell." The removal of non-functional microsomal enzymes and the SER aggregates with which they are associated could be necessary for the maintenance of a "metabolic equilibrium".

The more marked effect of CH_3HgCl on the pars recta cells of female rats in comparison to males probably is also due to sex differences in the activities of renal enzymes (55, 82, 83, 103, 104). The enzymes, 11β-hydroxysteroid dehydrogenase, 16-hydroxysteroid dehydrogenase, β-hydroxybutyrate dehydrogenase, acid phosphatase, alkaline phosphatase, glucose-6-phosphatase and nonspecific esterase, all show sex differences in renal activity. Alkaline phosphatase, acid phosphatase, β-hydroxybutyrate dehydrogenase, glucose-6-phosphatase and nonspecific esterase show sex differences in activity exclusively in the pars recta segments (83, 103, 104).

Enzymes which probably detoxify mercury may also show sex differences in activities. Because of such sex differences in enzyme activities, female proximal tubule cells would be unable to adequately detoxify mercury through conjugation with cysteine for excretion (108), thus allowing it to interact with cellular enzyme systems. The excretion of mercury-cysteine complexes in these cells may be less efficient than in male proximal tubule cells.

The concept that female rat proximal tubule cells are not able to detoxify and excrete mercury derived from methyl mercury is also supported by the presence of degenerating mitochondria and dense membranous cytosomes exclusively in pars recta cells. These cytosomes may represent a means for sequestering mercury. Lysosomes (cytosomes) from renal tubule cells concentrate cations and drugs *in vivo* (21, 22, 35, 36, 62, 87). Mercury derived from methyl mercury also accumulates in the lysosomal fractions of rat kidney and liver (68, 69). A number of investigators (22, 35, 36, 54) have isolated an acidic lipoprotein

component of lysosomes which is thought to be responsible for the binding of cationic compounds. The membranous dense appearance of the cytosomes observed in this investigation suggests a lipidic character. Large, dense membranous cytosomes have been observed (32) in proximal convoluted tubule cells of mice given the cationic herbicide paraquat. It was suggested that proliferation of acidic lipoprotein in response to paraquat resulted in the dense membranous appearance of the cytosomes. An analogous situation could occur in the pars recta cells of mercury-treated females if these cells were unable to sufficiently detoxify or excrete the mercury present. The cationic mercury might then stimulate production of the acidic lipoprotein component, giving rise to dense membranous cytosomes. The presence of these cytosomes together with degenerating mitochondria may be pathognomonic of mercury damage to pars recta cells.

The changes observed in the proximal tubule cells of male rats exposed to CH_3HgCl suggest that these cells are probably better able to detoxify mercury. The greater proliferation of SER in pars recta cells of males indicates that the cells respond better to the presence of CH_3HgCl than do pars recta cells of female rats. The previously noted sex differences in renal enzyme activities probably account for greater detoxification or secretory ability.

The numerous cytosegresomes containing mitochondria and microbodies observed in pars convoluta cells and the increased presence of degenerating pars recta cells in males given CH_3HgCl suggests that male proximal tubules are also affected. Several investigators (25, 27, 71,

(99) have noted increased cytosegresome formation in proximal tubule cells from animals treated with a number of compounds and suggested that it is indicative of a stress condition. The presence of mitochondria in cytosegresomes of pars convoluta cells from mercury-treated males may indicate that they are susceptible to mercury toxicity and that their turnover rate within the cells is accelerated.

This possibility may also be true for microbodies since numerous instances of microbody autophagocytosis and cytosegresome formation were observed. Hruban and co-workers (46, 47) have reported increased microbody autophagocytosis and turnover in cells of animals exposed to salicylates. The significance of microbody enzyme systems in CH_3HgCl metabolism is not known.

The apparent loss of SER-containing cytoplasmic masses in the urine may have some bearing on the increased protein excretion observed in persons occupationally exposed to organo-mercurials (51, 96).

In the present study, the glomeruli of all animals appeared structurally normal. No alteration of epithelial cell foot processes, basal lamina or capillary endothelium was noted. In view of the normal glomerular structure, it is unlikely that excessive amounts of protein are lost by this route. It may be that increased protein excretion is the result of proteins associated with cytoplasmic masses extruded from proximal tubule cells into the urine.

Animals exposed to both dieldrin and CH_3HgCl exhibited similar but less extensive cytologic changes than animals given either compound alone. This response was especially marked in female rat kidneys. The numbers of SER-containing cytoplasmic masses and dense membranous

cytosomes in the pars recta were greatly reduced. This may be attributed to stimulation of microsomal enzyme systems by dieldrin. Street *et al.* (88, 89, 90, 91, 92) have reported that administration of DDT to animals receiving dieldrin reduces tissue storage and enhances excretion of dieldrin. A potentiating effect of one toxicant on the metabolism of another is indicated. Dieldrin induction of microsomal enzymes in the female rat kidney could also increase the metabolism of CH_3HgCl. This concept is reinforced by the decreased numbers of cytosegresomes and degenerating cells observed in male animals. Proximal tubule cells of these animals were similar to controls except for the presence of small SER aggregates. These aggregates may morphologically represent the effect of dieldrin induction of microsomal enzymes.

Dieldrin-Induced Arteritis

The arteritis observed in histologic sections of kidneys from both male and female dieldrin-treated rats was not observed in either control or mercury-treated animals. This arteritis was also noted in lung arteries of dieldrin-treated animals (R. E. Brooks, unpublished results). Harr *et al.* (41) have described a similar arteritis in the brains of rats given low doses of dieldrin for long periods. The young age of the animals used in this study and the presence of an intact tunica intima in affected arteries suggest that the arteritis is not the polyarteritis known to occur in old Wistar rats (76).

The mechanism by which dieldrin could produce an arteritis is not understood, but two major possibilities exist. If dieldrin has a toxic effect on cells of the vessels, then cellular destruction would initiate

an inflammatory response. A second possibility is that dieldrin or its hydrophilic metabolite 6,7-trans-dihydrodihydroxy-aldrin could have conjugated with serum protein or the acidic surface coat of artery wall cells (31, 38, 60) as a hapten. This possibility is supported by the observed presence of eosinophils in the inflammatory exudates. These cells are known to be attracted by the presence of antigen-antibody complexes (9).

In conclusion, this study indicates that chronic exposure to low doses of the environmental toxicants produces morphologic alterations to kidney tubules which are detectable by electron microscopy. In mature animals of good nutritional status, the extent of these changes in the proximal tubules seems largely dependent on the sex. The concept that one toxicant may influence the toxicity of another is important because humans and animals are exposed simultaneously to numerous environmental toxicants.

The lack of increased pathologic changes in animals given dieldrin and/or CH_3HgCl for longer time periods suggests that the detoxification enzyme systems of the proximal tubule cells reach a "steady state" condition in the presence of low level doses of these chemicals. The cells are apparently able to perform their normal metabolic functions while adapting to the constant presence of these compounds.

The ultimate effect of these toxic chemicals on animal longevity or their effect on other organ systems cannot be predicted on the basis of these morphological studies.

SUMMARY AND CONCLUSIONS

This investigation was undertaken to evaluate the morphologic effects of chronic exposure to low doses of the pesticide dieldrin and methyl mercuric chloride (CH_3HgCl) on rat kidney.

Kidney tissue was studied by light and electron microscopy from rats that received 5.0 ppm dieldrin, 2.0 ppm CH_3HgCl, or 5.0 ppm dieldrin plus 2.0 ppm CH_3HgCl in their diets for 84 or 142 days.

Histologic and ultrastructural changes were confined to the proximal tubules. Alterations in these tubules were consistent for each regimen but did not progress with duration of exposure. Female rats were more markedly affected than males. In general, the convoluted portion of the proximal tubule (pars convoluta) reacted differently to dieldrin and methyl mercuric chloride than the straight segment of the tubule (pars recta).

Proximal tubule cells of male and female rats exposed to 5.0 ppm dieldrin in the diet showed an increase of smooth endoplasmic reticulum (SER), degenerating mitochondria and tubule cell death. Male rats showed a greater apparent increase in SER than did females. Some female rats displayed prominent vacuolation of the proximal tubules in response to dieldrin.

Proximal tubule cells of animals given 2.0 ppm CH_3HgCl in the diet also exhibited increased amounts of SER, degenerating mitochondria and cell death. The pars convoluta cells of female rats were less markedly affected than those of the pars recta. Pars recta tubules were dilated and contained within the lumens many spherical, hematoxylin staining

cytoplasmic masses which were visible by light microscopy. These masses were characterized ultrastructurally by the presence of an SER aggregate. In addition, cells of the pars recta contained electron-dense membranous cytosomes not present in control animals. Cells of the pars convoluta of male rats displayed increased numbers of autophagic cytosegresomes. Pars recta cells of males showed an increase in SER, but the dense membranous cytosomes observed in the pars recta cells of female rats were not seen.

Rats exposed to 5.0 ppm dieldrin plus 2.0 ppm CH_3HgCl showed less morphologic alteration of the proximal tubules than animals of the previous group, but greater amounts of SER and more degenerating proximal tubule cells were observed in comparison to control animals. The incidence of SER-containing cytoplasmic masses and dense membranous cytosomes observed in the pars recta of females from this group was greatly reduced in comparison to CH_3HgCl-treated females. Male rats showed fewer autophagosomes and degenerating mitochondria in proximal tubule cells than did males exposed only to CH_3HgCl.

These findings are discussed in relation to sexual differences in renal enzyme activities and induction of microsomal enzyme systems by dieldrin. Proliferation of SER in proximal tubule cells of dieldrin-treated rats was discussed in relation to induction of microsomal enzymes associated with the SER. The prominent effect of methyl mercury on the pars recta segments of female rats was attributed to the conversion of CH_3HgCl to Hg^{++} *in vivo* and the known toxicity of Hg^{++} to the pars recta. Sex differences in detoxification enzyme activities probably accounted for the more marked effect of CH_3HgCl on female

proximal tubules in comparison to those of male rats. The reduced effect of dieldrin plus CH_3HgCl on proximal tubules may be explained by dieldrin induction of detoxification enzymes.

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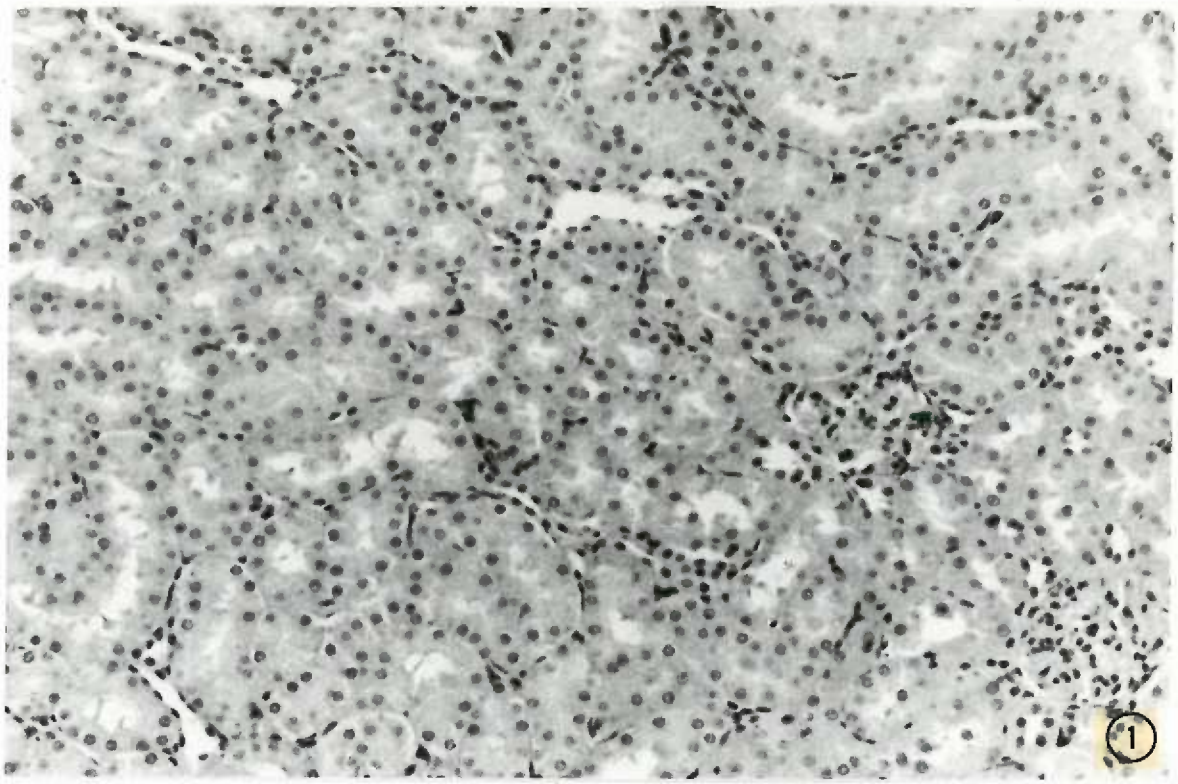
Figure 1.

Light micrograph of a kidney section from a 142 day female control rat. This section is representative of renal cortex from control animals. X 220.

Figure 2.

Higher magnification of Figure 1 illustrating appearance of proximal tubule pars recta segments from control animals. X 880.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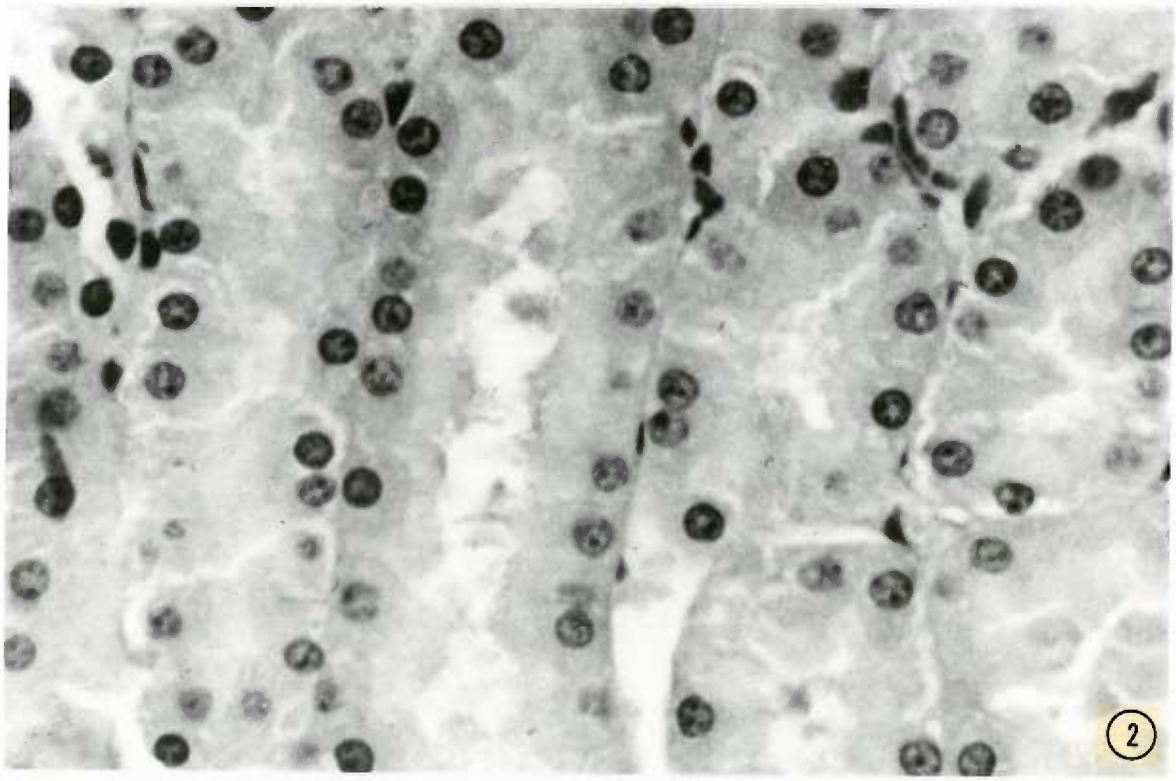
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Figure 3.

Electron micrograph of typical proximal tubule (pars convoluta) cell from an 84 day female control animal displaying microvilli (MV) of brush border, cytosomes (c) and basal interdigitations (arrow). X 15,400.

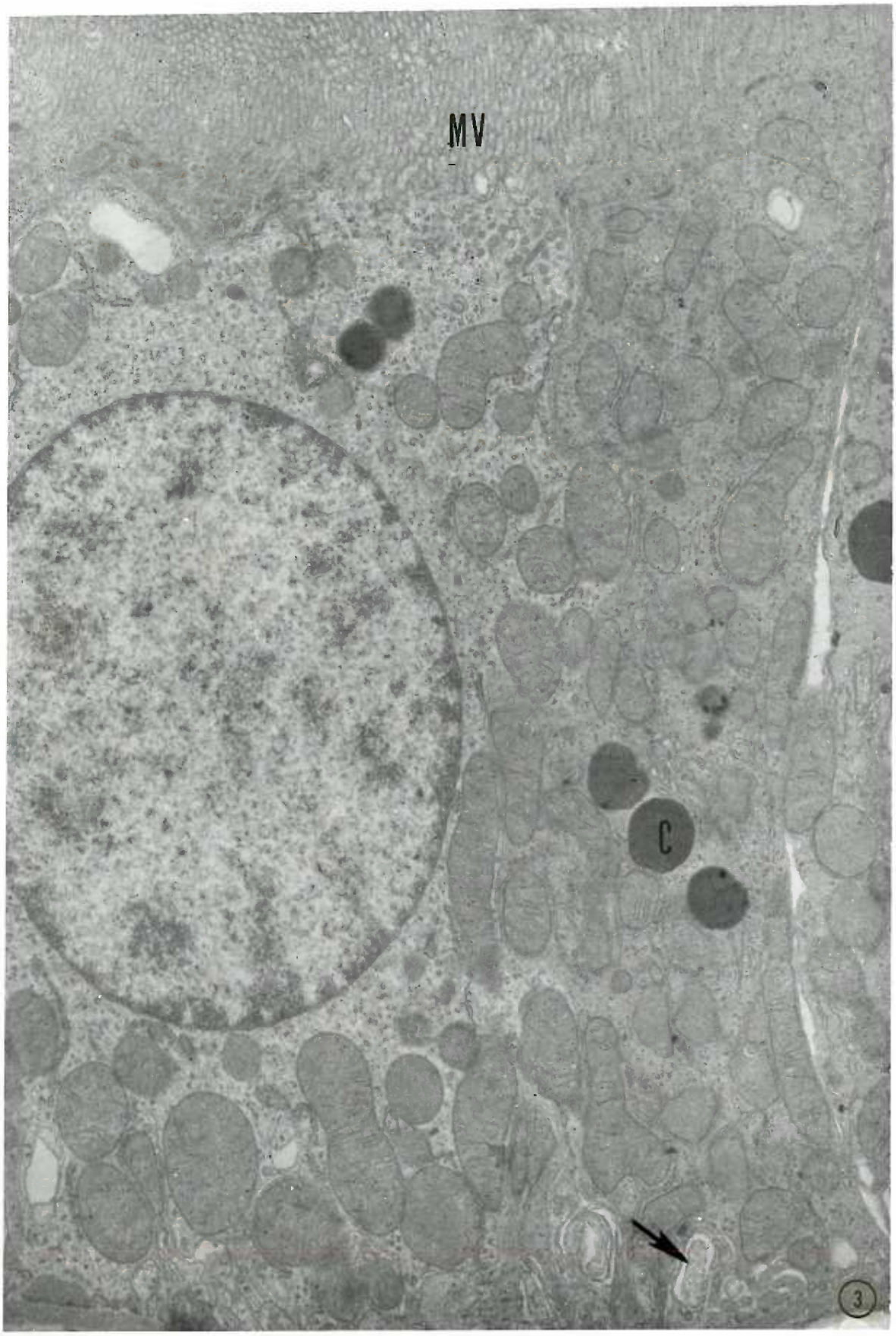
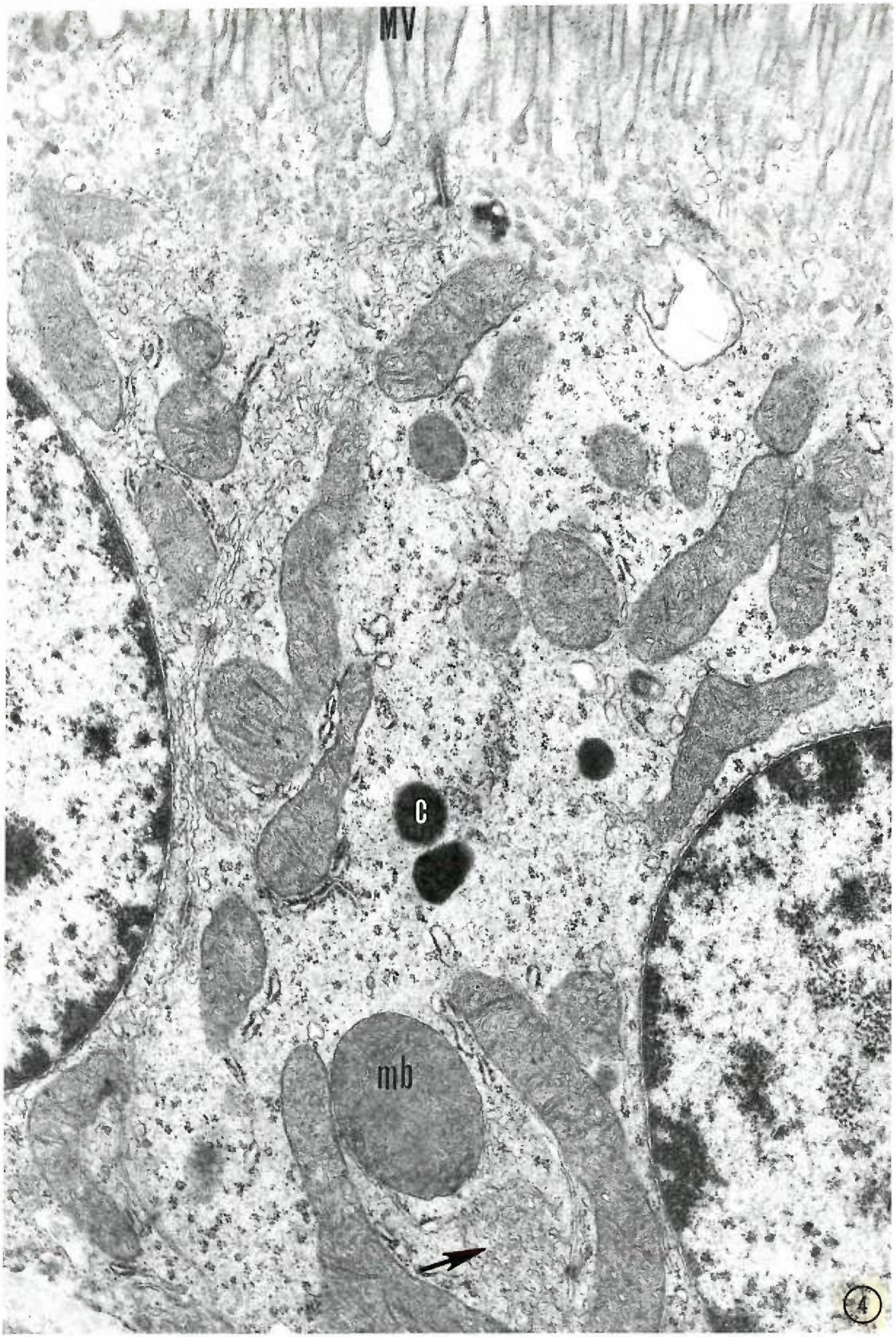

MV
C
3

Figure 4.

Pars recta cell from an 84 day female control showing low cuboidal profile, microvilli (MV), cytosomes (c), microbody (mb) and small aggregates of SER (arrow). X 20,500.


MV
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mb
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Figure 5.

Light micrograph of renal cortex from a male rat receiving dieldrin for 84 days, exhibiting architecture indistinguishable from control renal cortex. X 220.

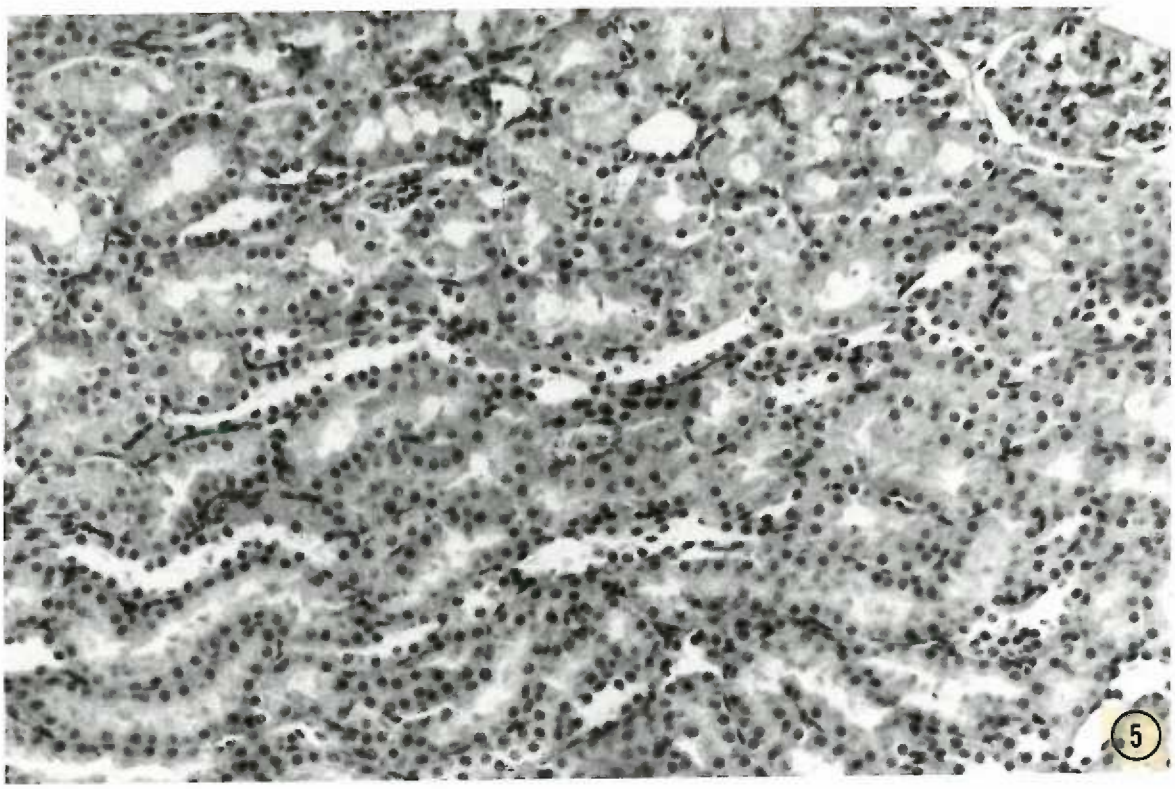
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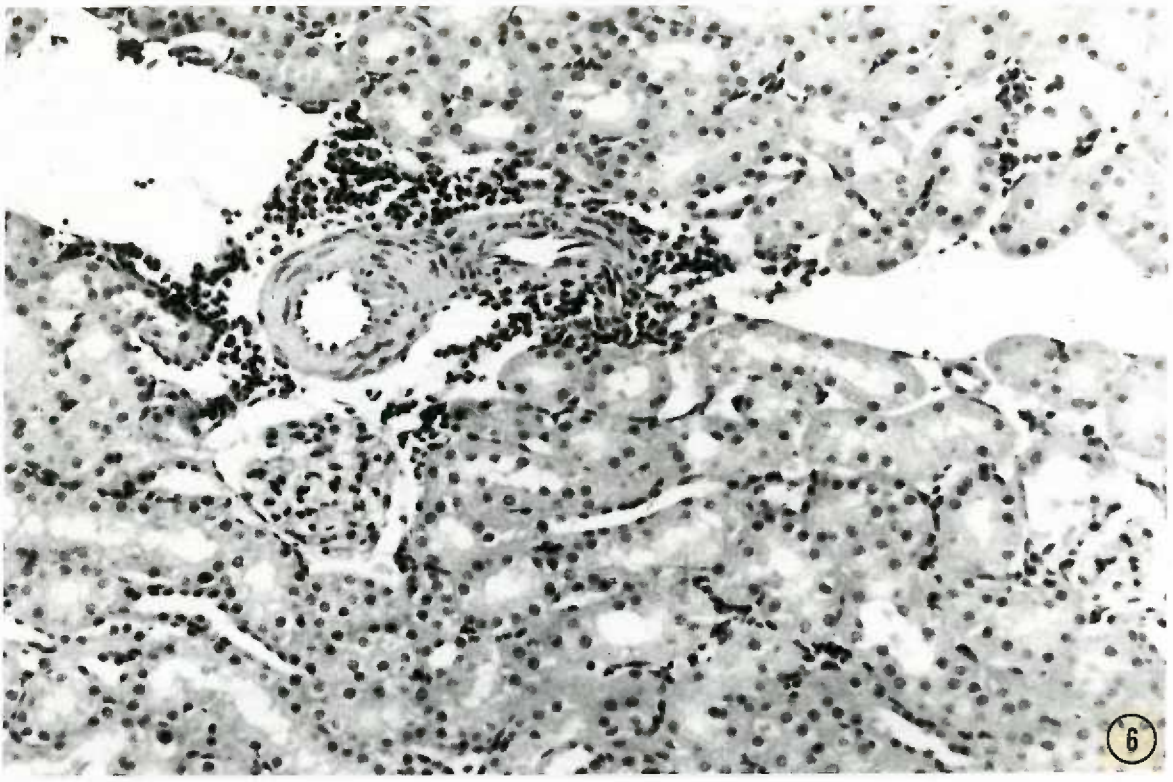
Figure 6.

Arteritis observed in 142 day dieldrin treated female rat characterized by inflammatory exudate. X 220.

Figure 7.

Higher magnification of same slide showing cells of inflammatory exudate. Neutrophils and lymphocytes predominate, but eosinophils are also prominent. X 880.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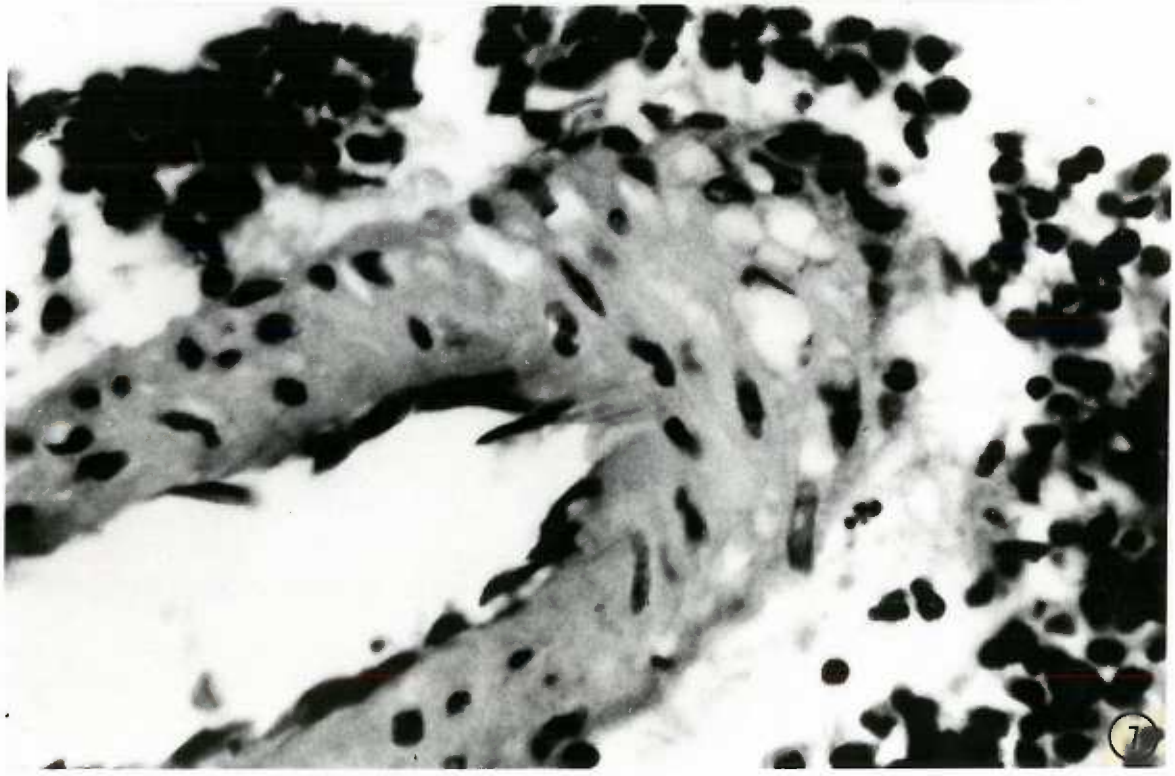
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Figure 8.

Pars convoluta from a female rat that received dieldrin for 84 days, showing degenerating mitochondria and SER bundle (arrow). X 15,400.

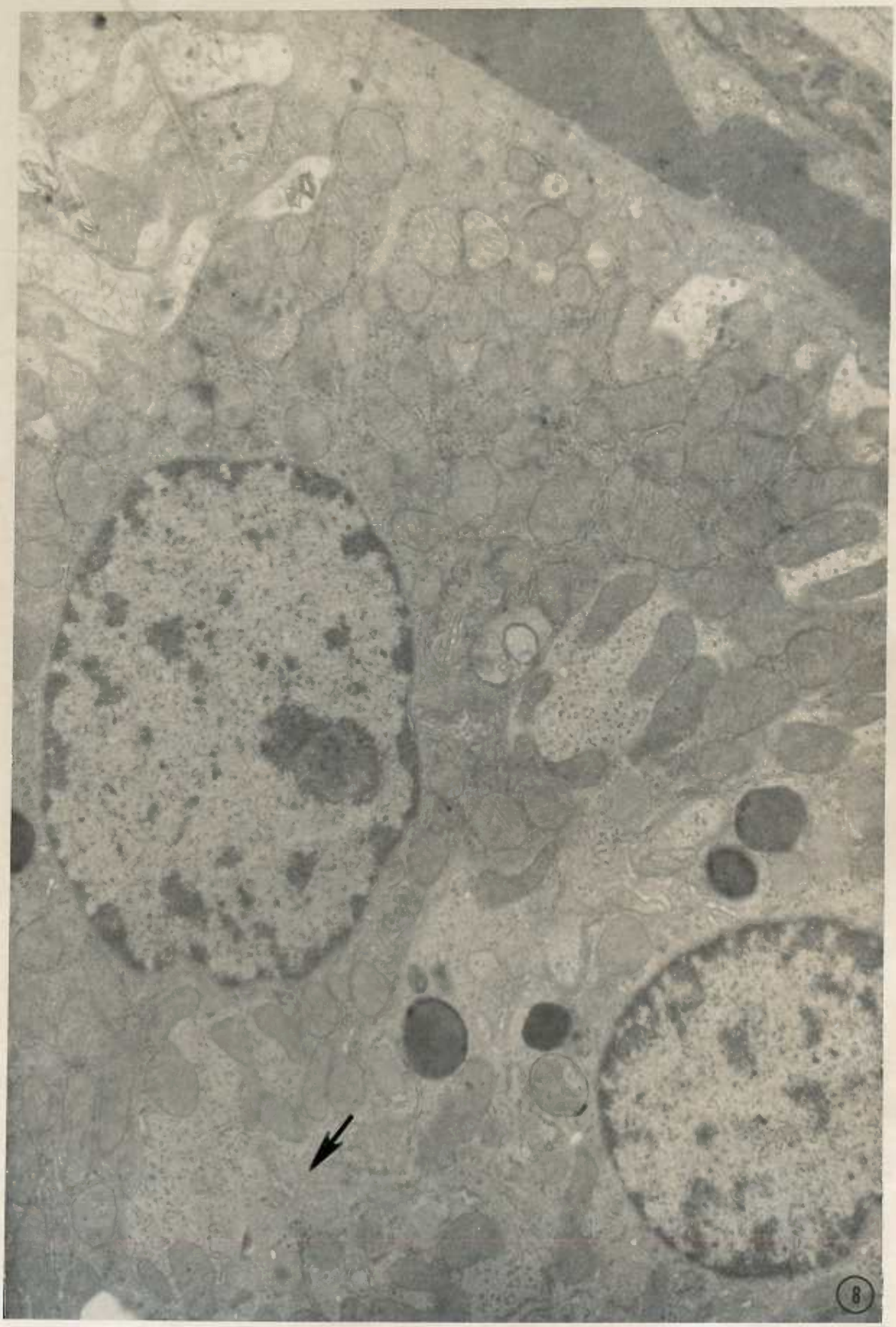
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Figure 9.

Female rat given dieldrin for 84 days. Pars recta cell displaying vacuoles containing flocculent lipidic material and scattered ribosomes. X 20,500.

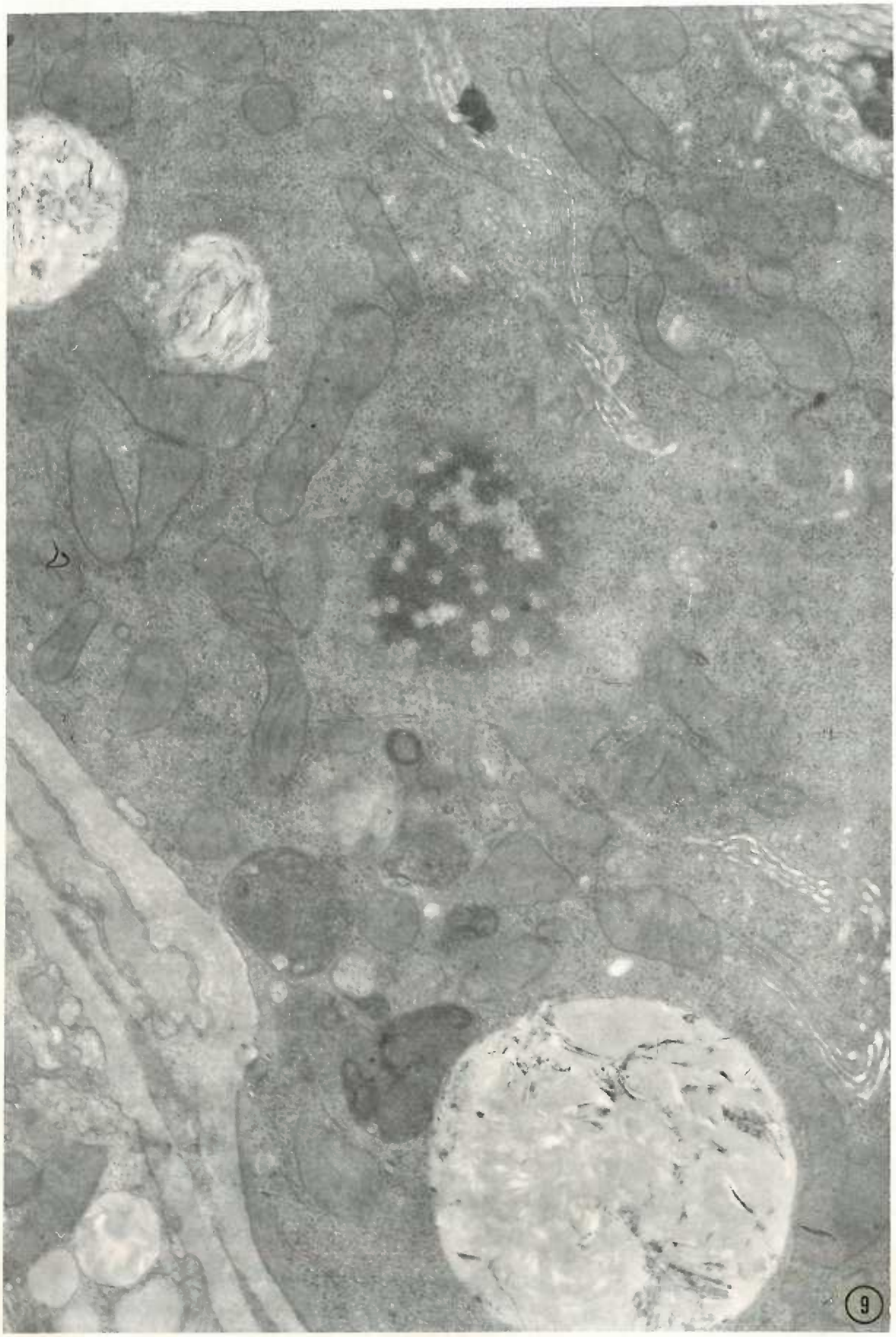
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Figure 10.

Pars convoluta cell from a male rat exposed to dieldrin for 84 days. Note presence of SER aggregates. X 14,850.

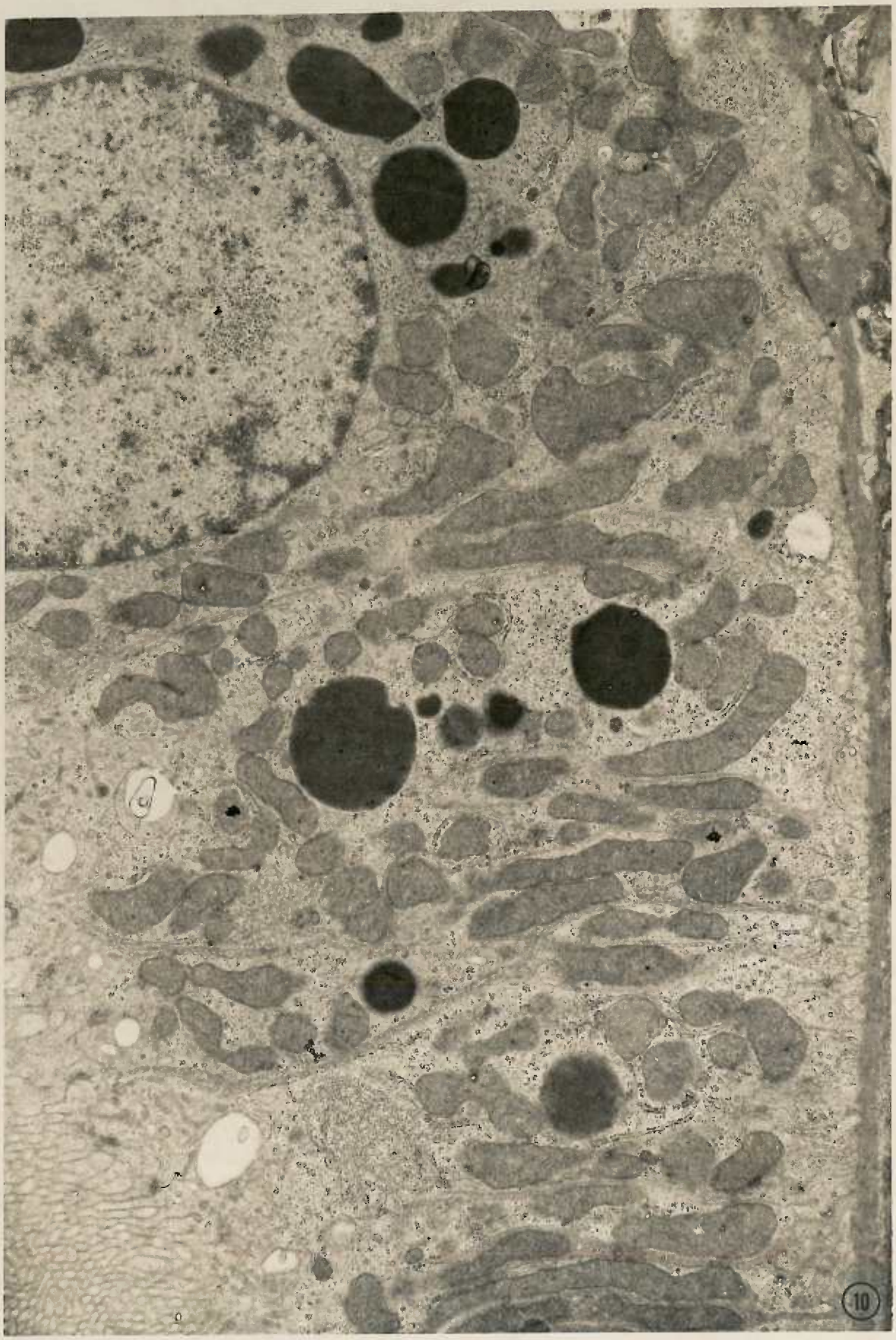
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Figure 11.

Pars convoluta cells from a male fed dieldrin for 142 days. These cells contain swollen degenerating mitochondria and SER aggregates. X 20,500.

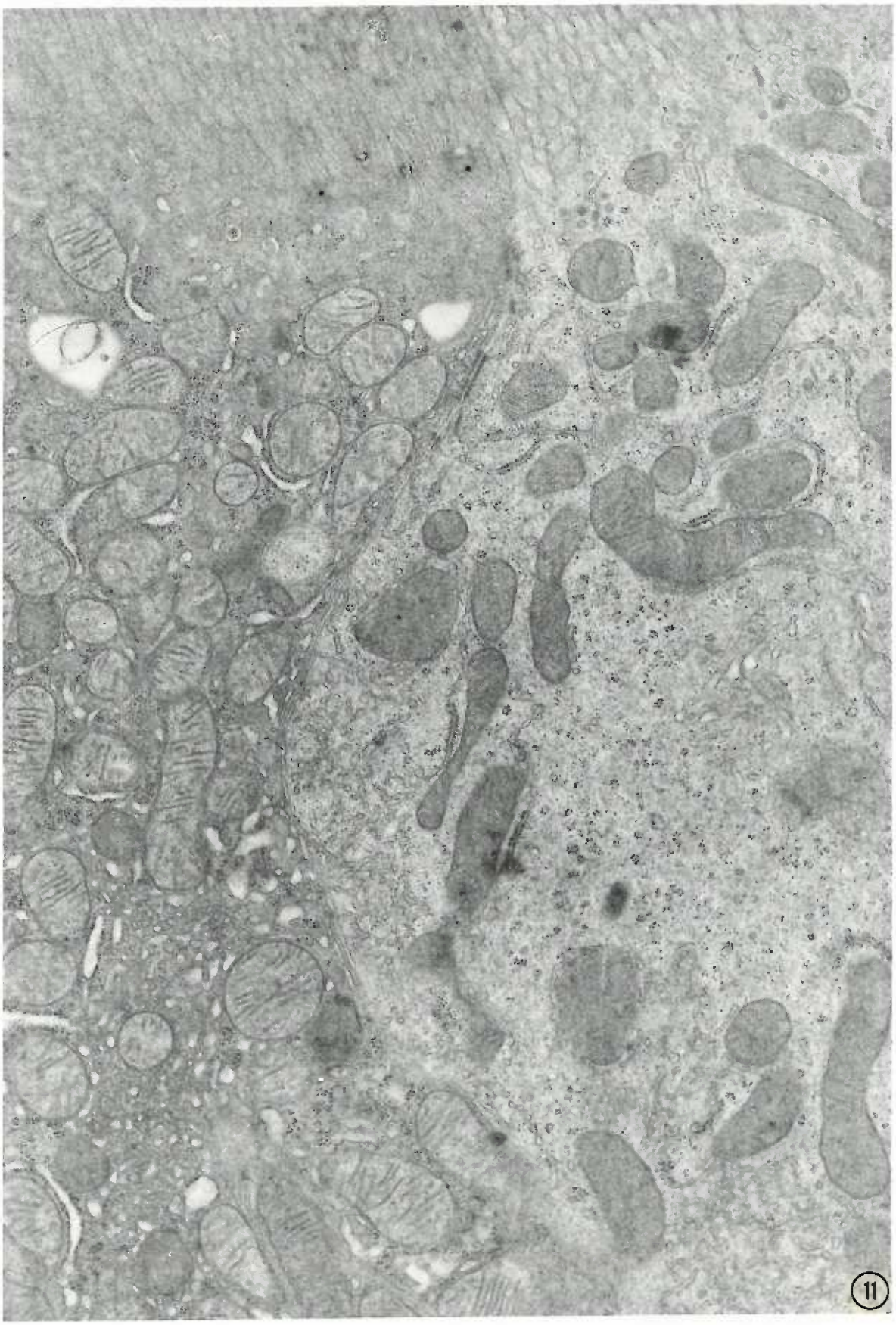

11

Figure 12.

Pars recta cells from a male rat fed dieldrin for 142 days showing numerous SER aggregates. X 20,500.

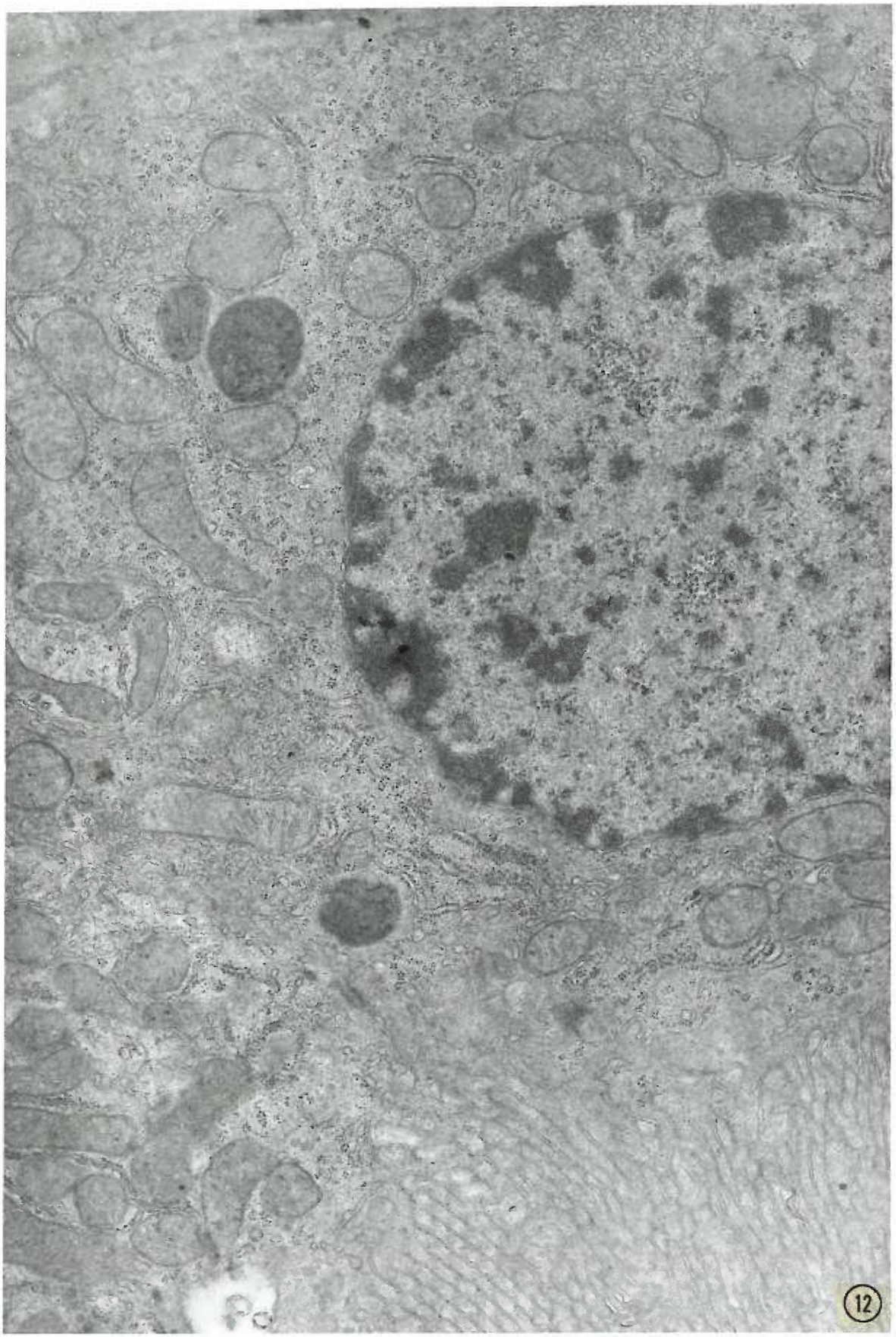
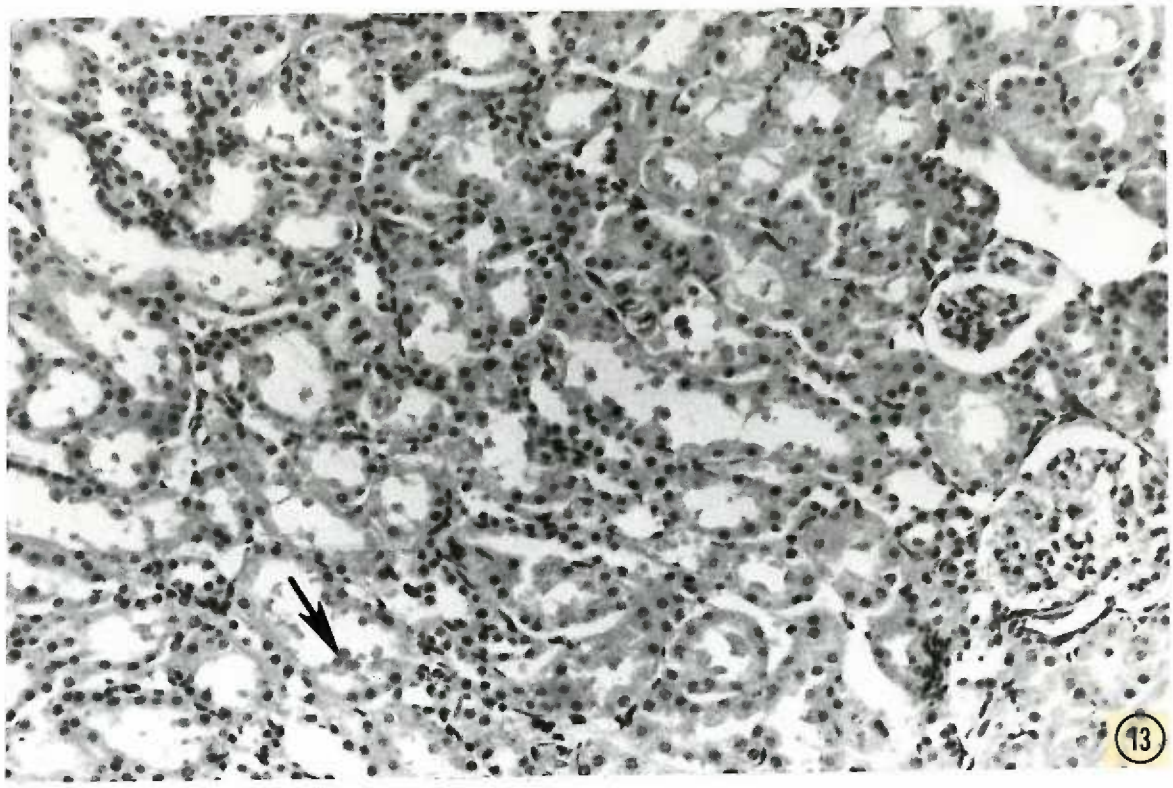

12

Figure 13.

Inner renal cortex from a female rat exposed to CH_3HgCl for 84 days. The pars recta segments exhibit marked dilatation and numerous spherical masses within the tubule lumens (arrow). X 220.

Figure 14.

Higher magnification of Figure 13 illustrating apical cytoplasmic swelling of pars recta cells (arrow) and bleb-like spherical masses in lumens. X 880.



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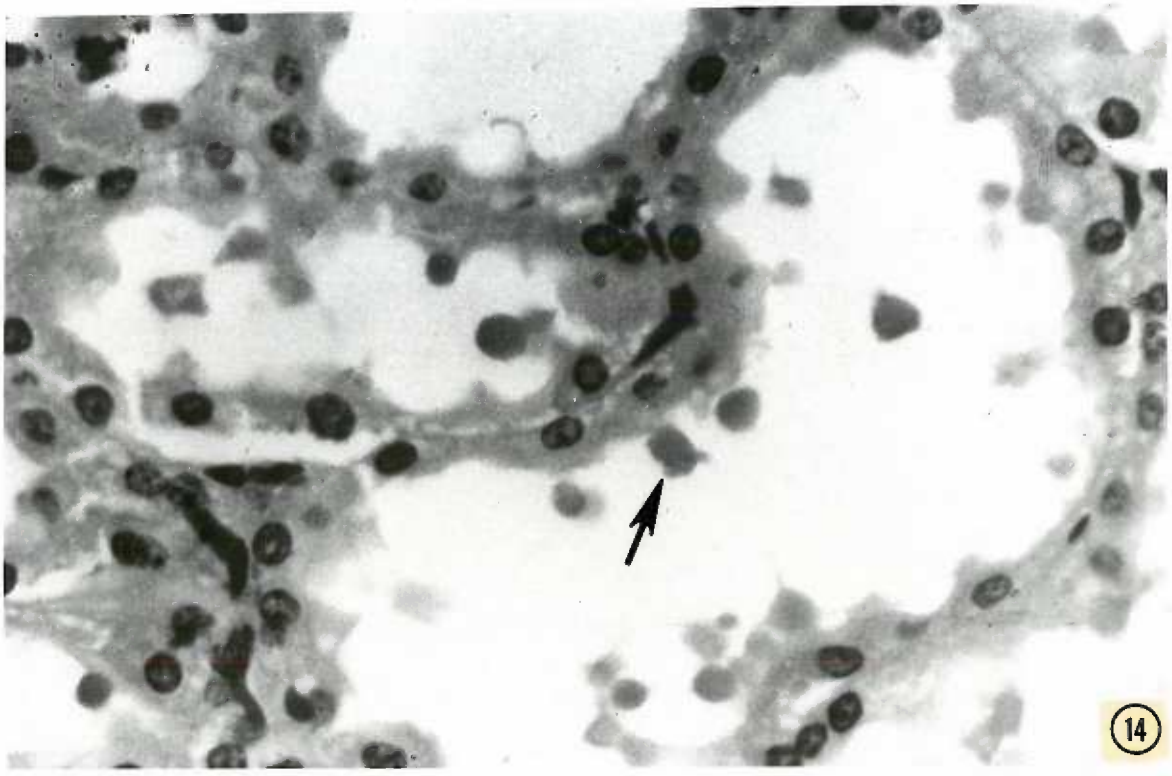


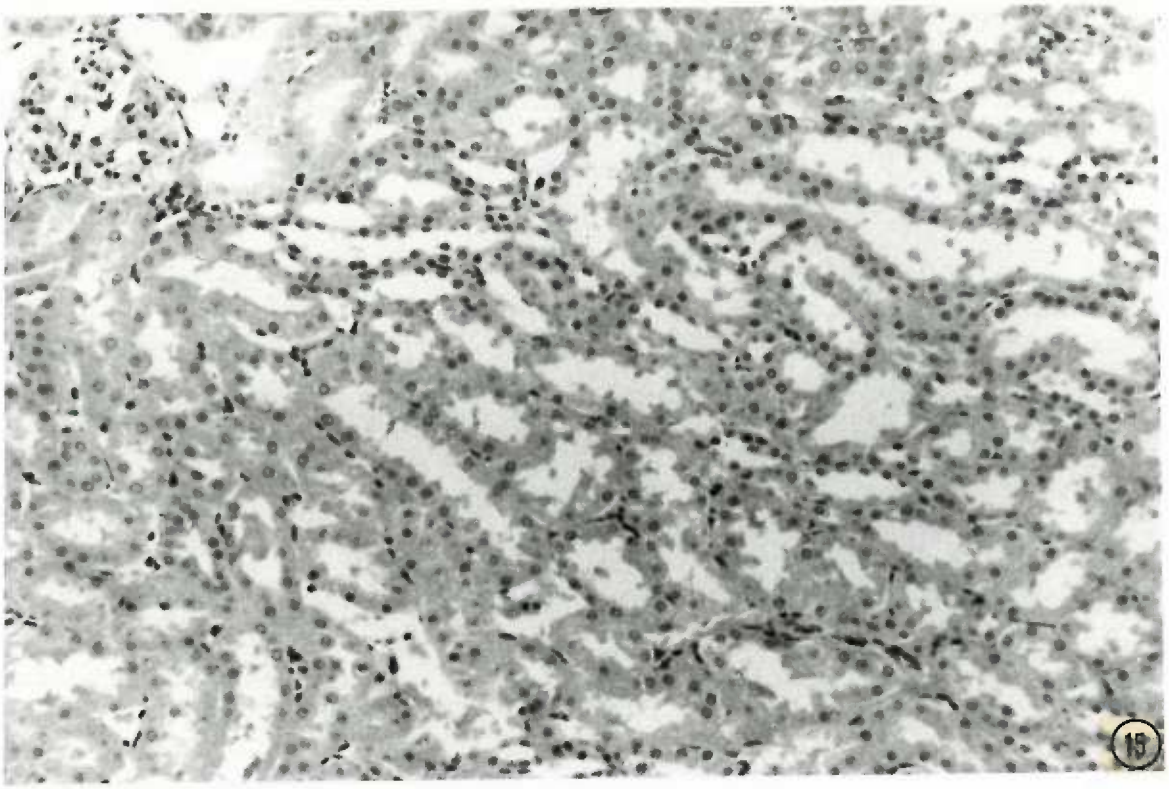
14

Figure 15.

Light micrograph of renal cortex of a female rat given CH_3HgCl for 142 days. This micrograph shows dilatation of pars recta segments and spherical masses in lumens. X 220.

Figure 16.

Higher magnification of Figure 15 with pars recta cells displaying apical cytoplasmic swelling and spherical masses in tubule lumens. X 880.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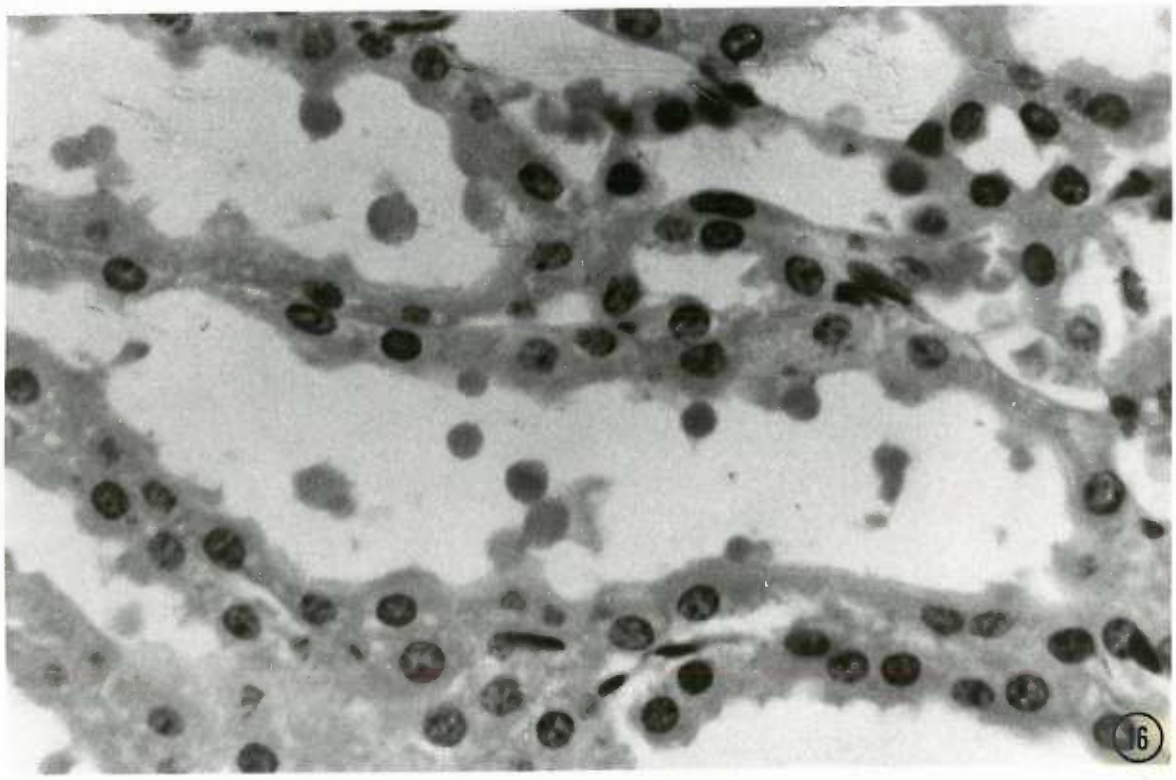

16

Figure 17.

Cell of the pars convoluta segment from a female rat given CH_3HgCl for 84 days exhibiting a vacuole and architecture otherwise similar to controls. X 18,500.

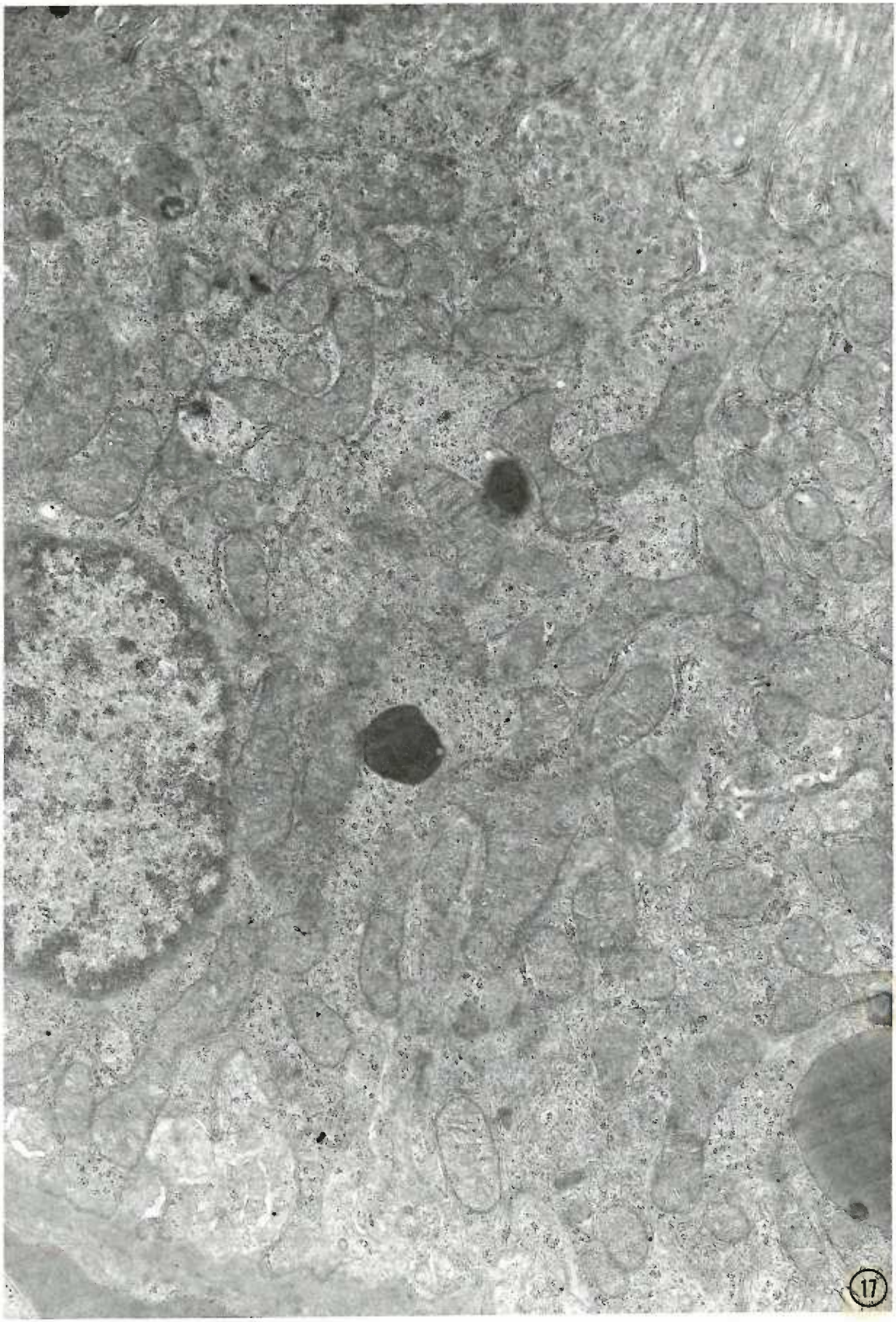

17

Figure 18.

Cells of the pars recta from the kidney of a female rat receiving CH_3HgCl for 84 days. Note the presence of spherical cytoplasmic masses containing SER in the patent lumen. SER aggregates are prominent in the cytoplasm. X 11,055.

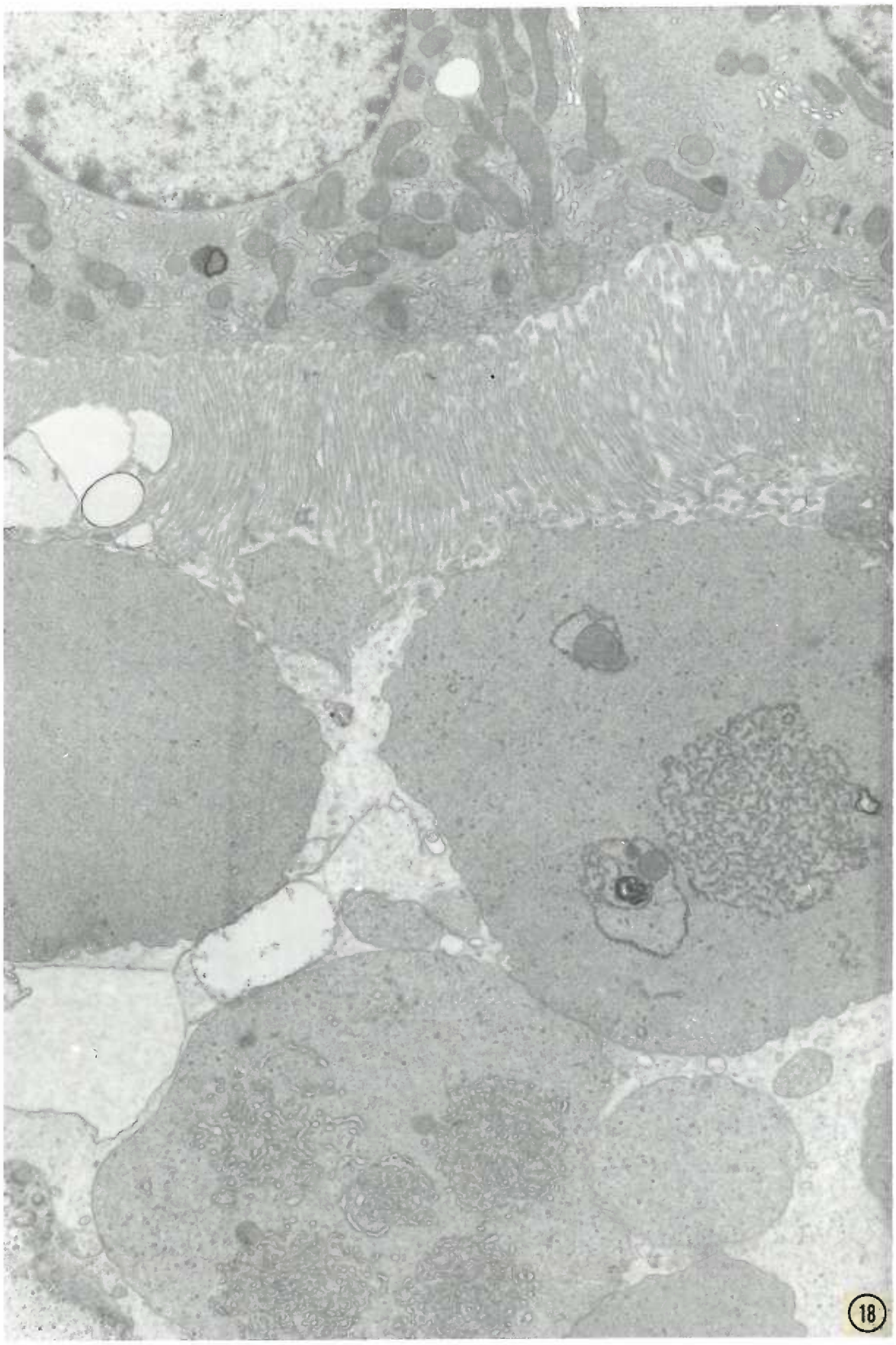

18

Figure 19.

Pars recta cell from a female rat given CH_3HgCl for 84 days showing an isolated spherical mass containing SER in the apical portion of the cell. X 14,500.

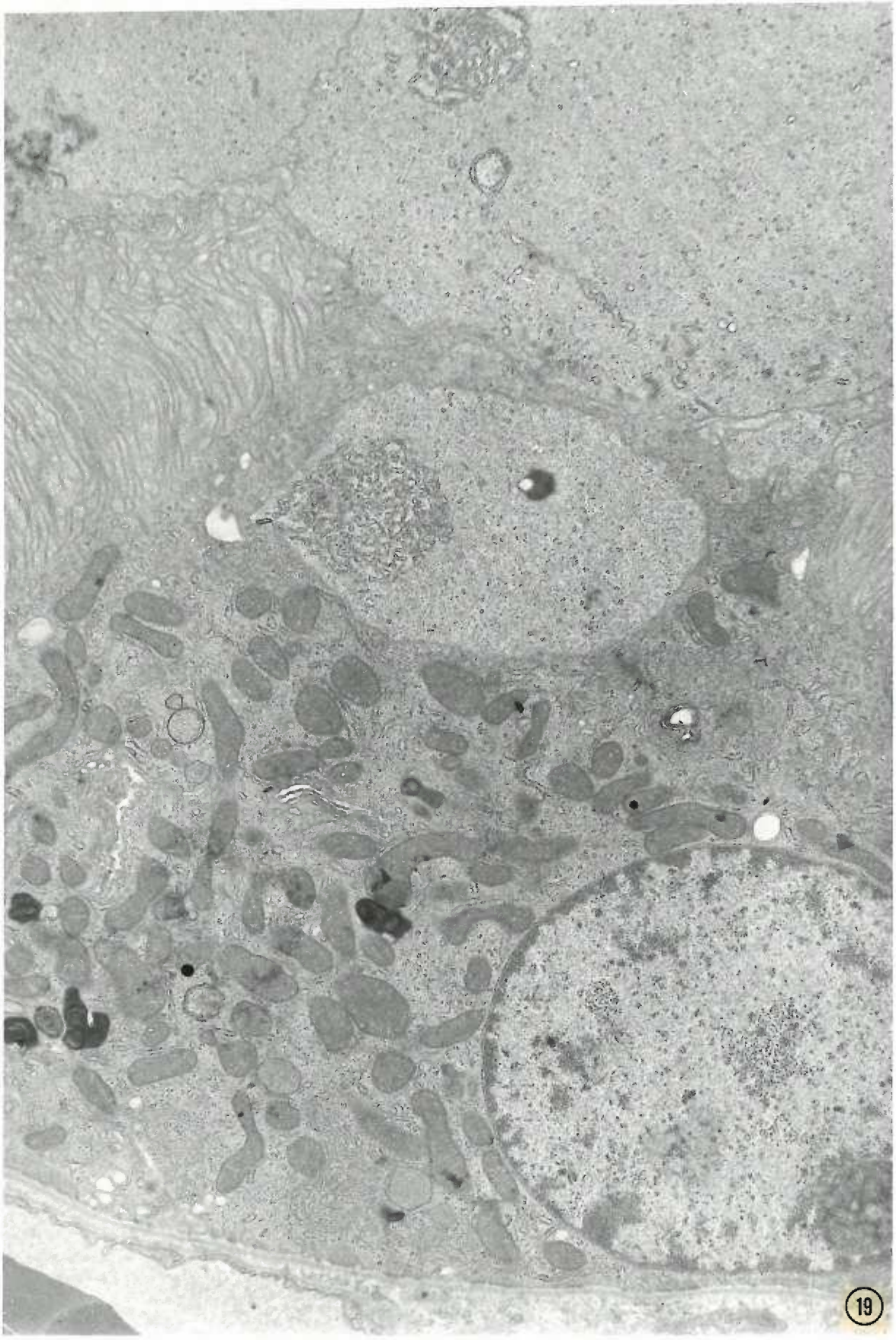
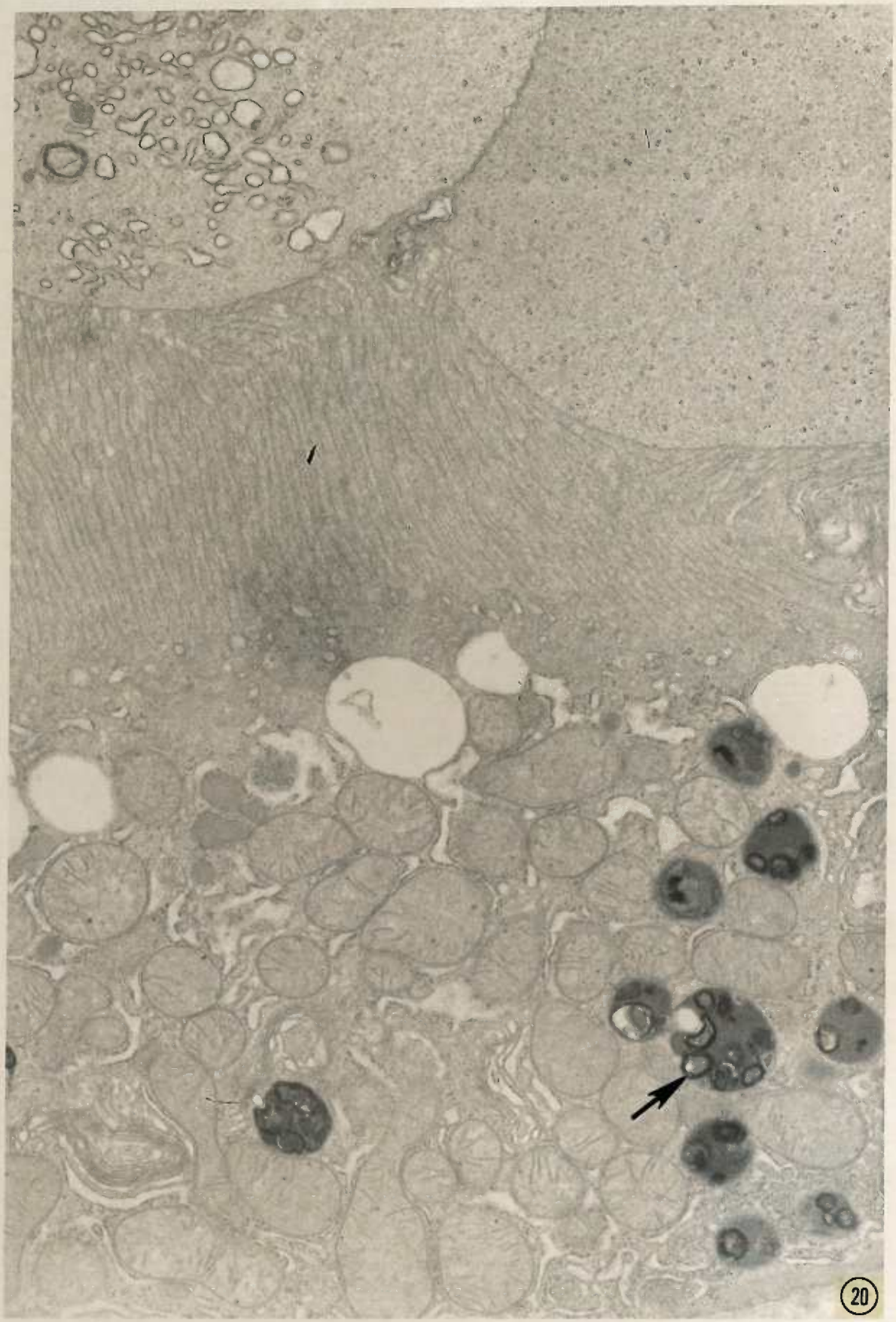

19

Figure 20.

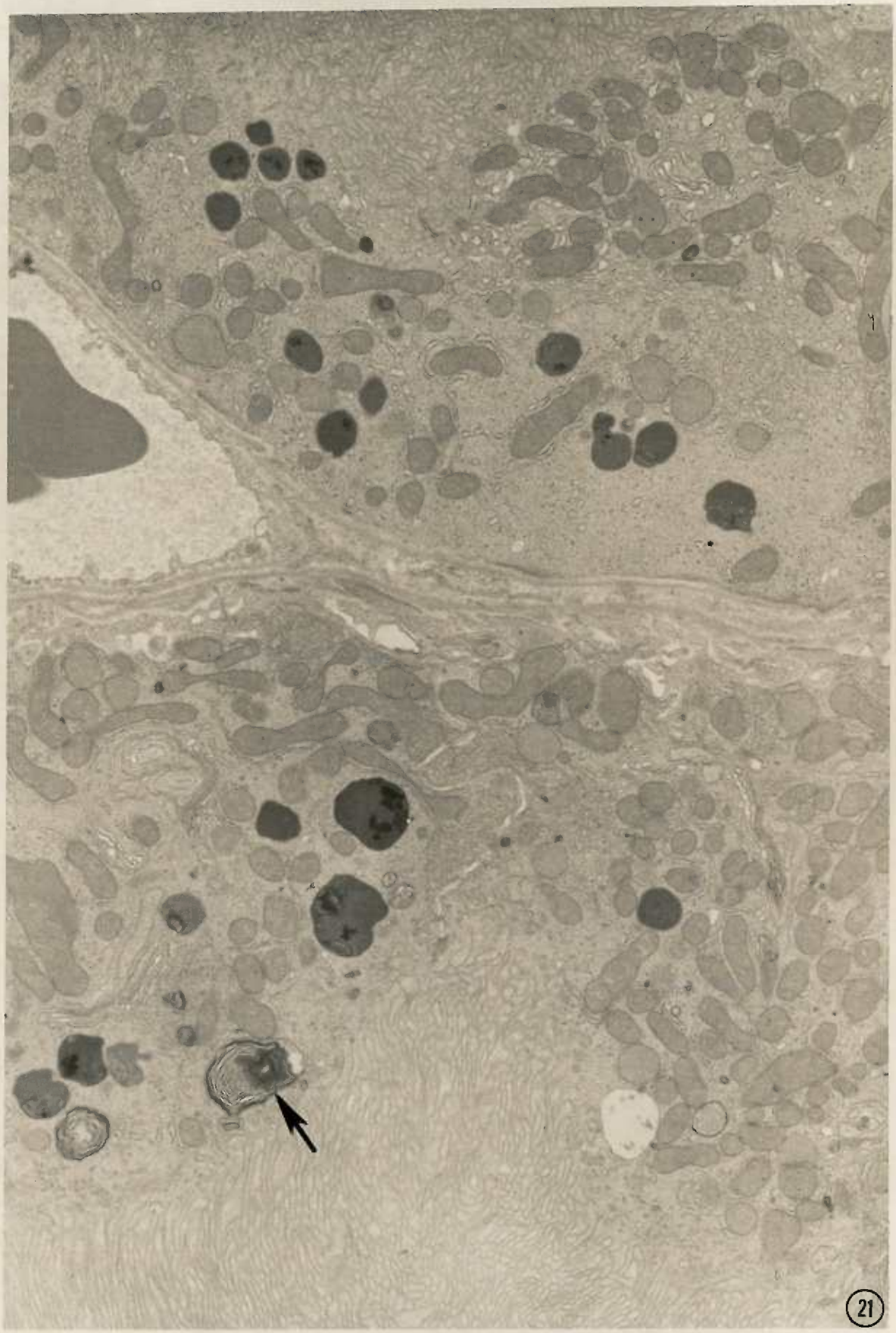
Dense membranous cytosomes (arrow) and swollen mitochondria in pars recta cell of a female rat exposed to CH_3HgCl for 84 days. Ribosomes and SER profiles are prominent in cytoplasmic masses present in tubule lumen. X 18,500.



20

Figure 21.

Pars recta segments of a female rat given CH_3HgCl for 84 days illustrating membranous dense cytosomes and myelin-like residual bodies (arrow). SER aggregates are also prominent. X 11,745.



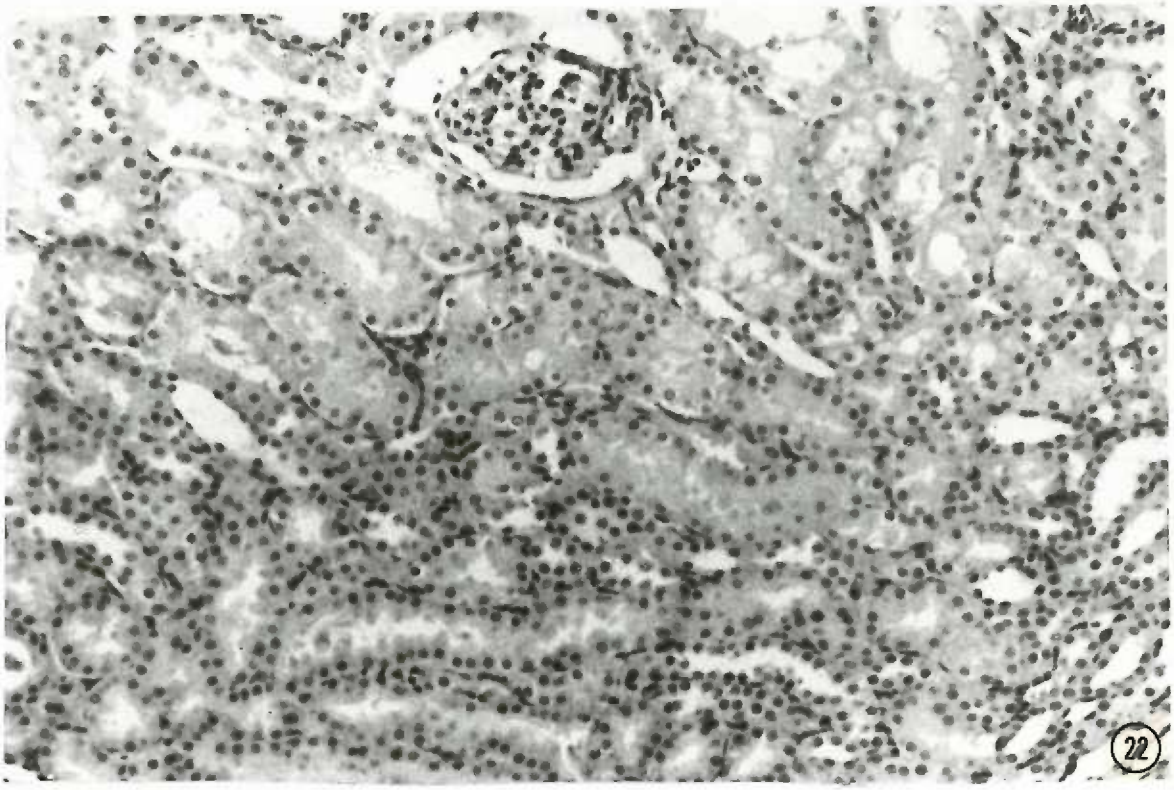
21

Figure 22.

Light micrograph of inner renal cortex from a male rat given CH_3HgCl for 84 days showing appearance similar to controls. X 220.

Figure 23.

Higher magnification of Figure 22 illustrating typical appearance of pars recta segments. X 880.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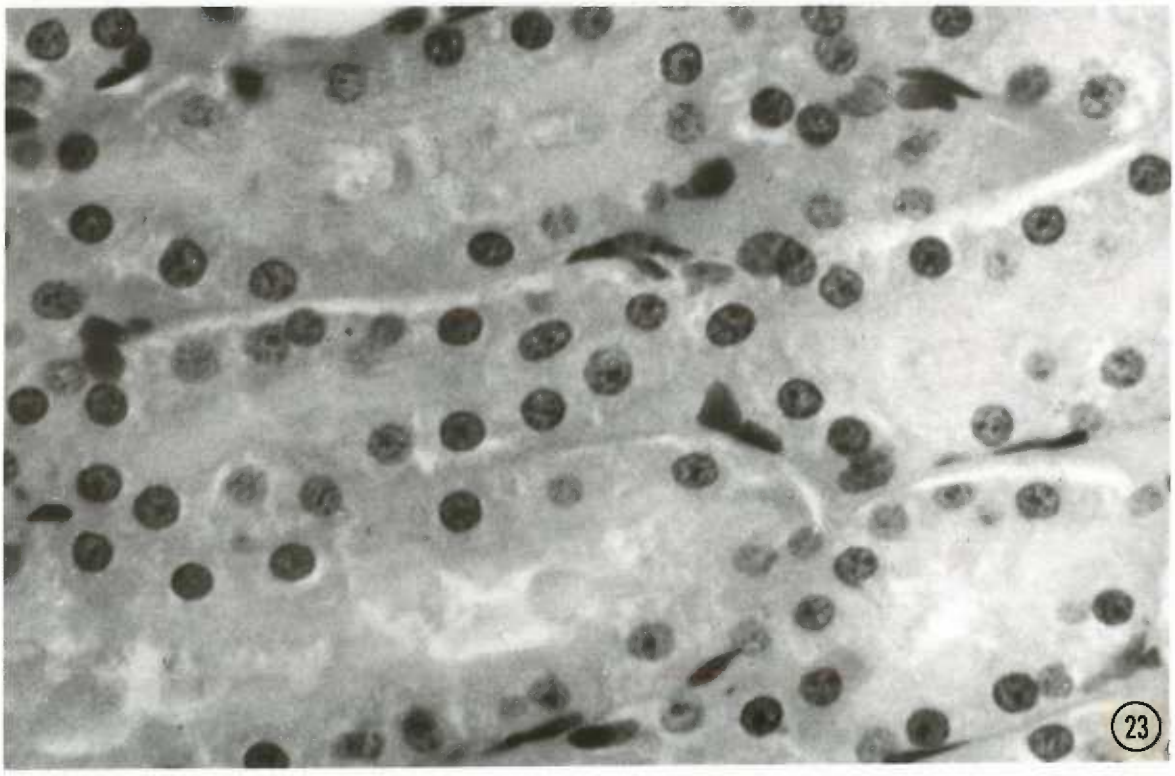

23

Figure 24.

Pars convoluta cells from a male rat given CH_3HgCl for 84 days showing degenerating cell between two viable cells. X 14,850.

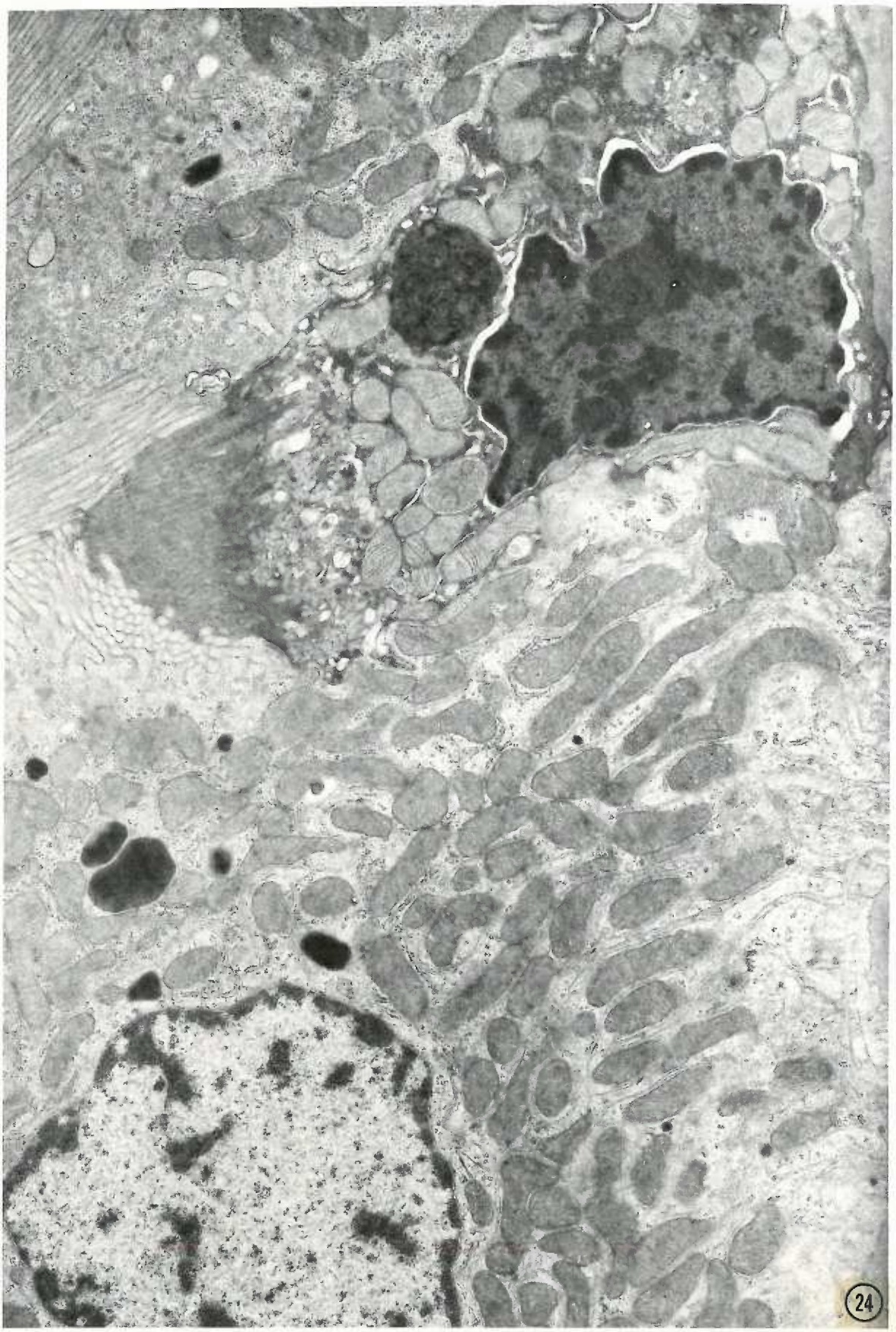

24

Figure 25. Several autophagic cytosegresomes from a pars convoluta cell of a male rat given CH_3HgCl for 84 days. X 39,500.

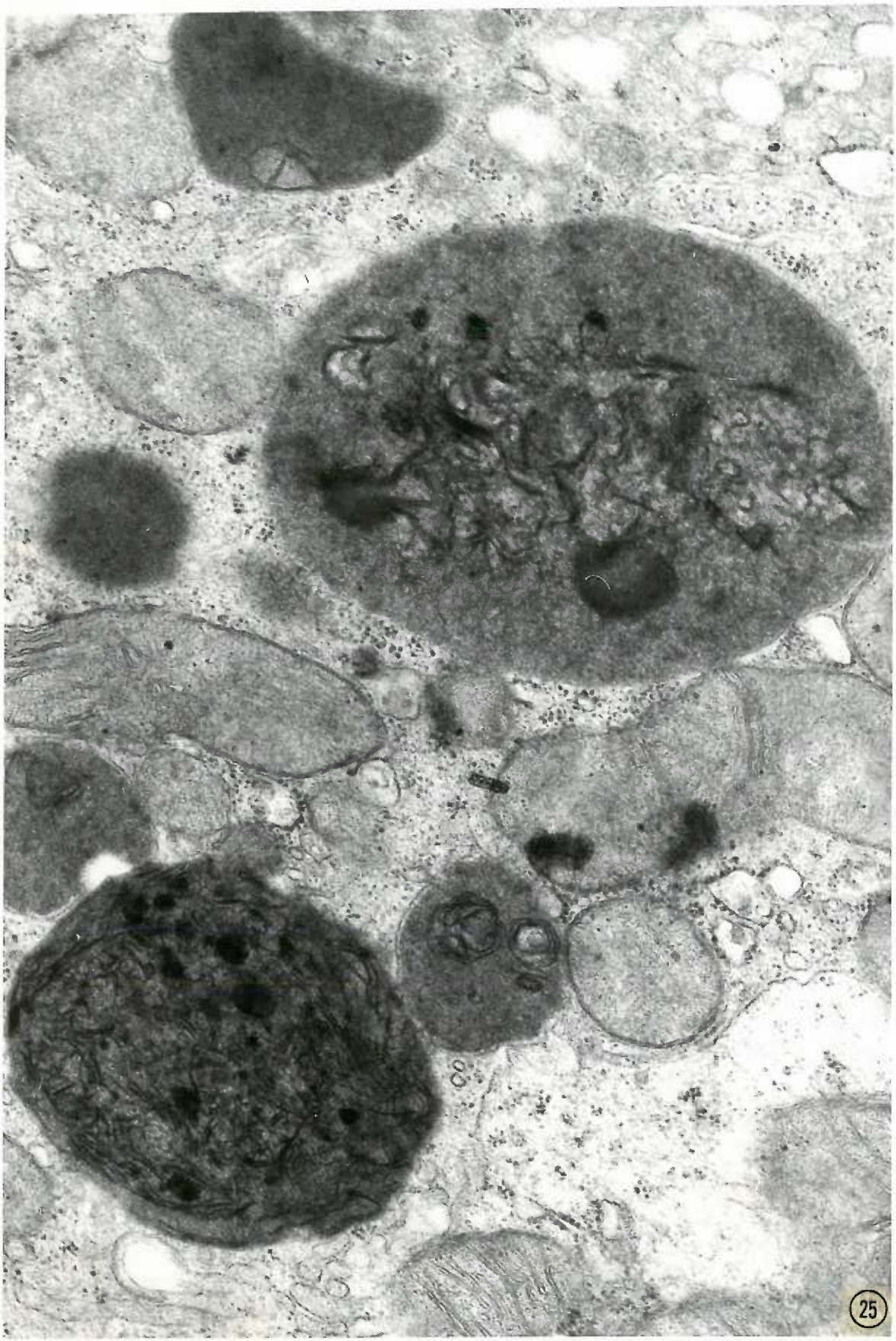

25

Figure 26.

Autophagic cytosegresome and several microbodies almost entirely enveloped by endoplasmic reticulum (arrows). Pars recta cell from a male rat given CH_3HgCl for 84 days. X 28,500.

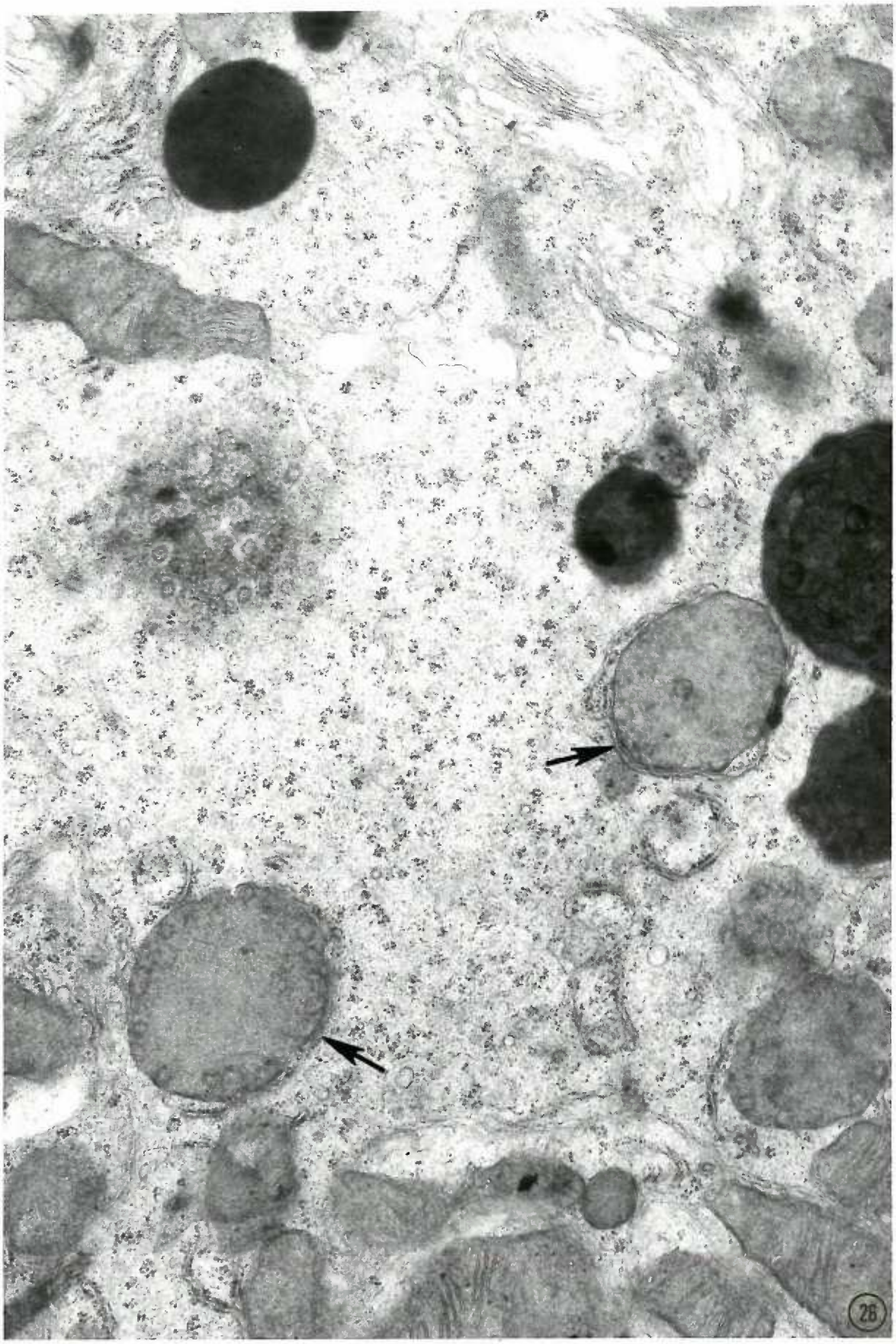

26

Figure 27.

Pars convoluta cell from a male exposed to CH_3HgCl for 84 days, displaying autophagic cytosegresome and a microbody completely isolated from remainder of the cytoplasm (arrow). X 28,500.

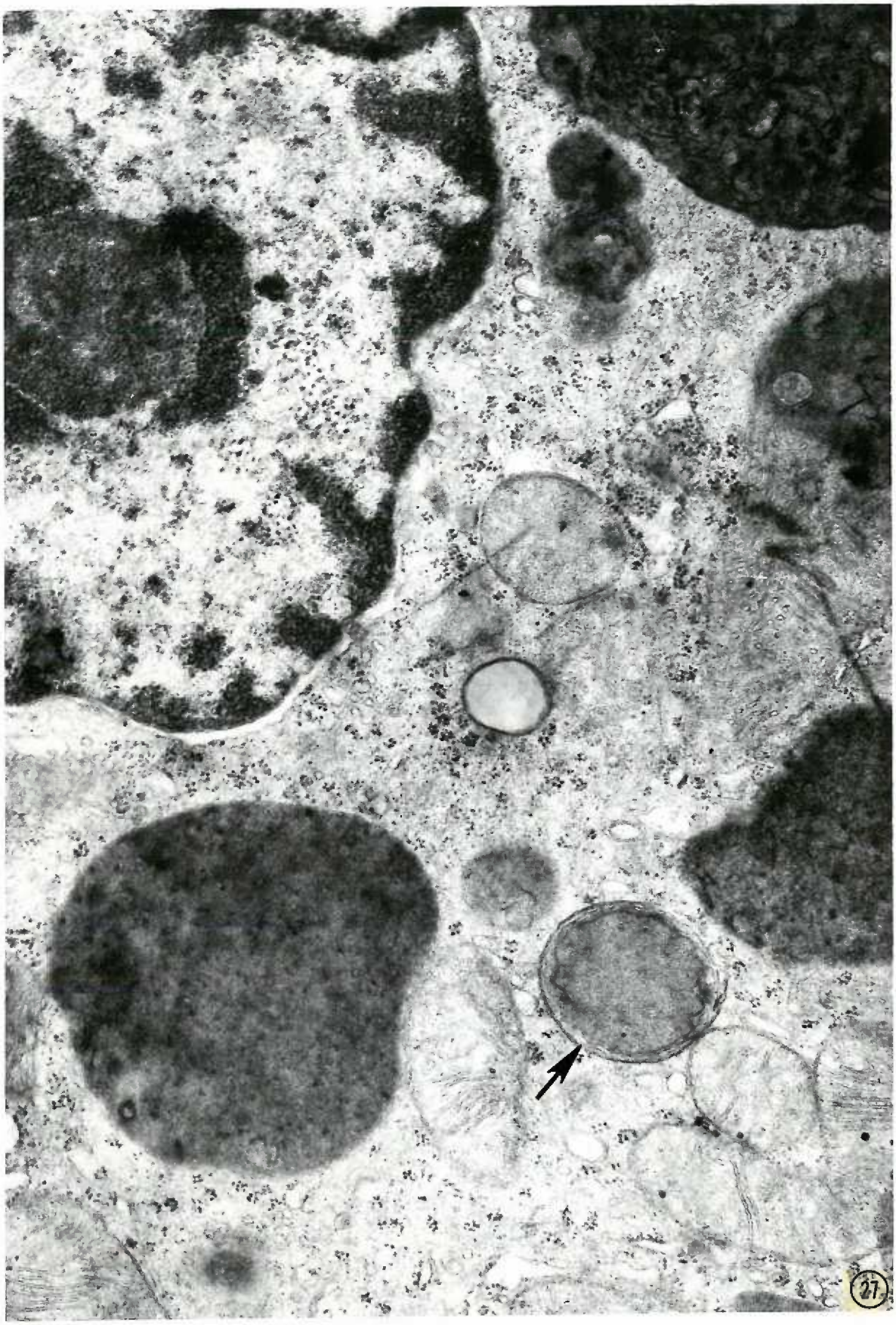

27

Figure 28.

Pars recta cell from a male administered CH_3HgCl for 84 days. Note presence of vacuoles, cytosegresomes (arrows) and small aggregate of SER. X 20,500.

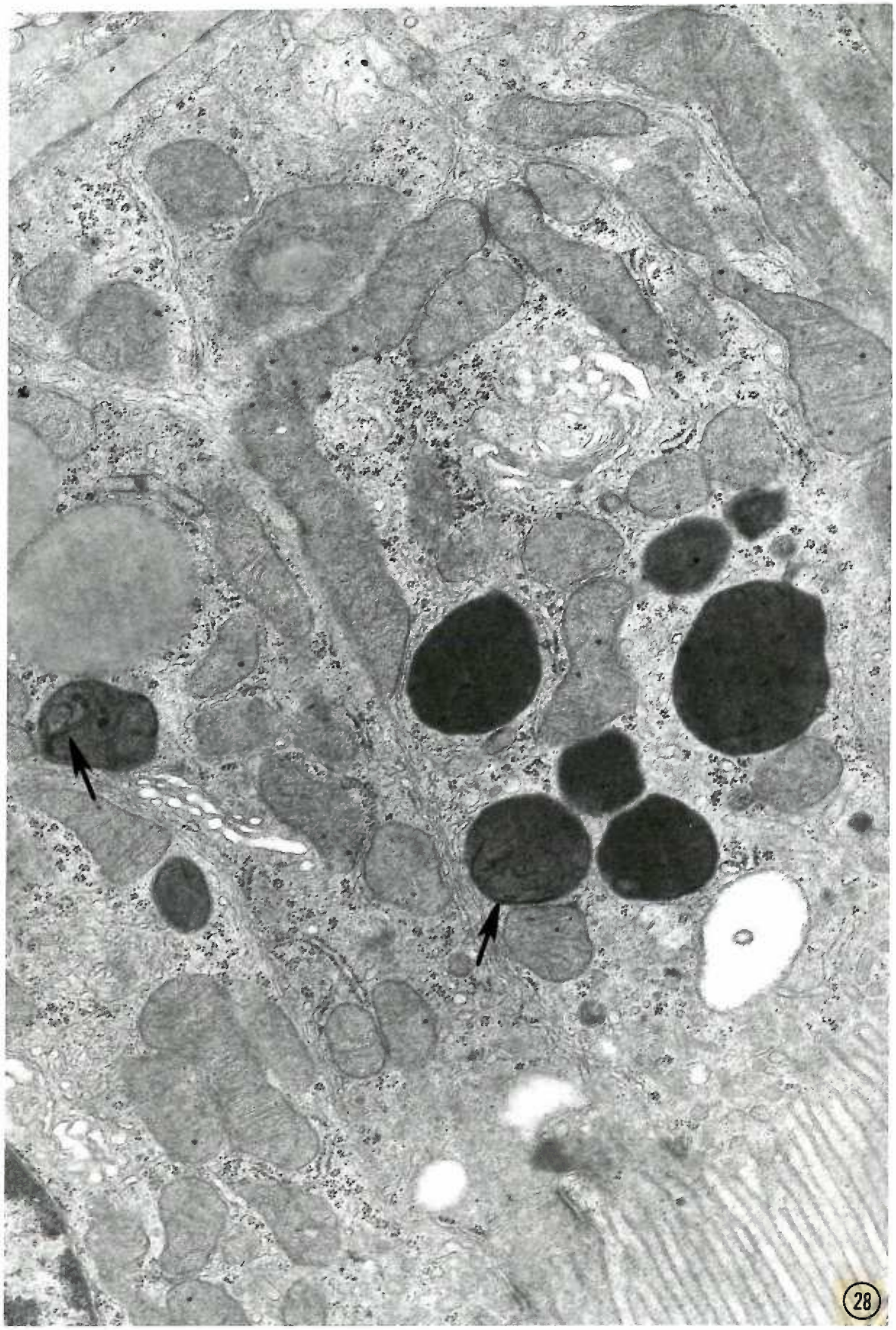
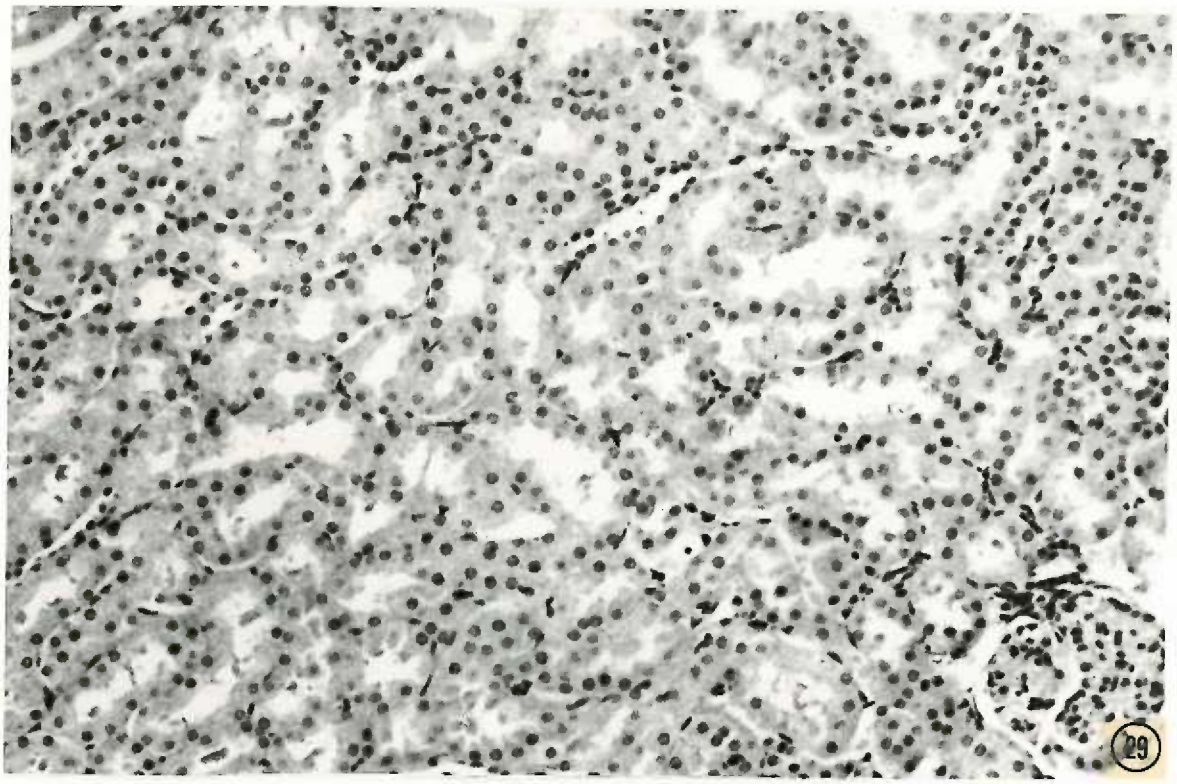

28

Figure 29.

Light micrograph of inner renal cortex of a female rat exposed to dieldrin plus CH_3HgCl for 84 days. The pars recta segments are less dilated and contain fewer spherical masses in tubule lumens than animals receiving CH_3HgCl alone. X 220.

Figure 30.

Higher magnification of Figure 29 showing one of the few tubules containing cytoplasmic masses. X 880.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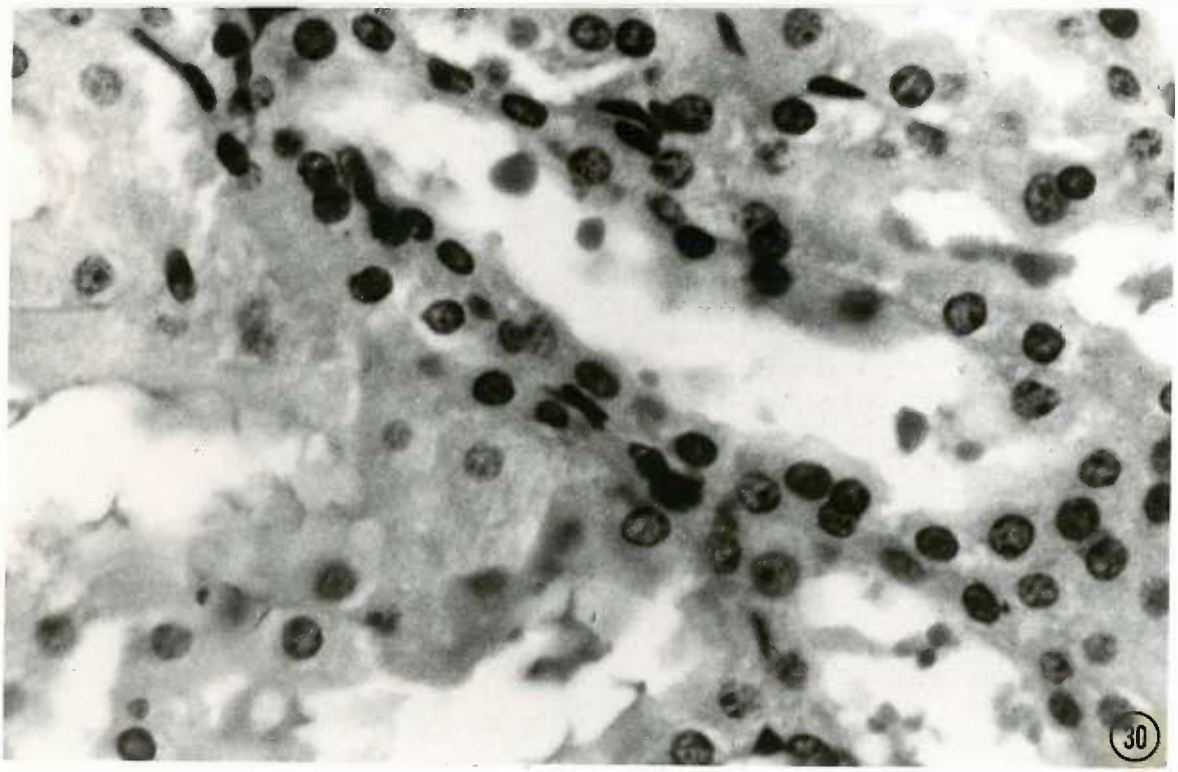
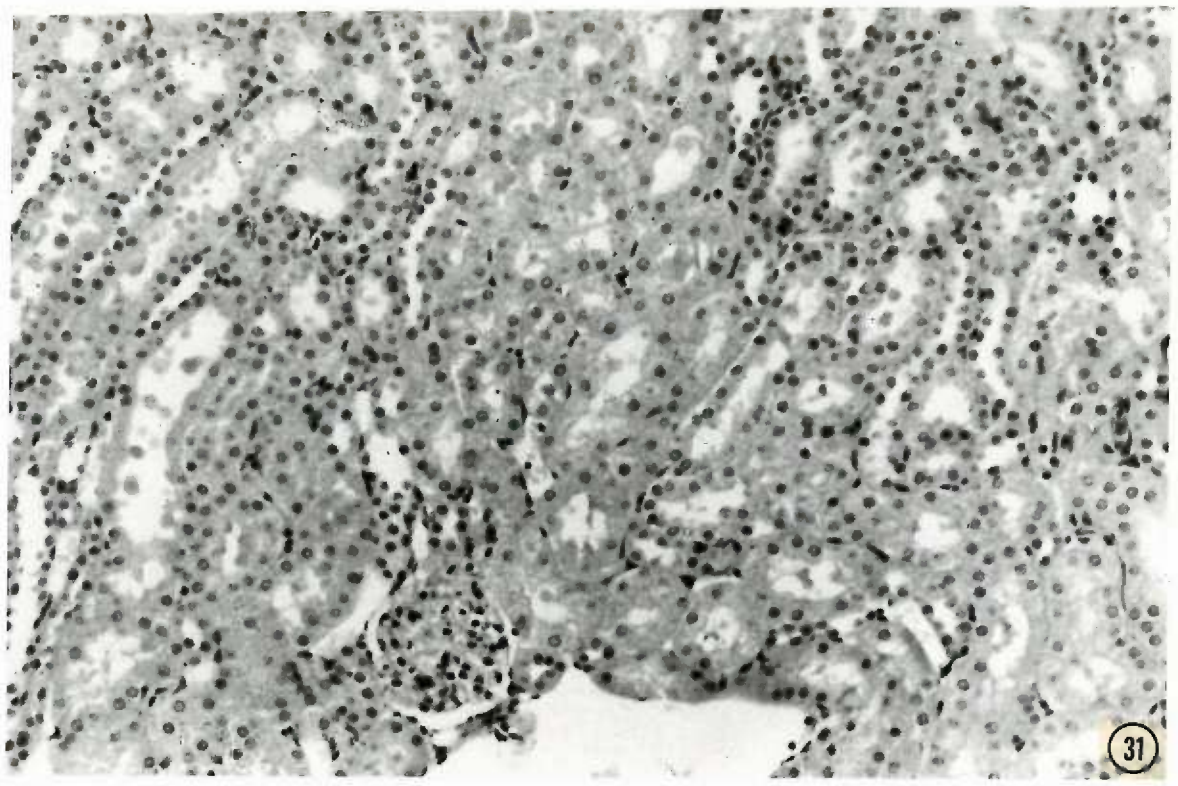

30

Figure 31.

Renal cortex of female rat given dieldrin plus CH_3HgCl for 142 days. Less dilatation and fewer spherical masses are evident in the tubule lumens in comparison to CH_3HgCl females. X 220.

Figure 32.

Higher magnification of Figure 31 illustrating one of the few tubules containing cytoplasmic masses. X 880.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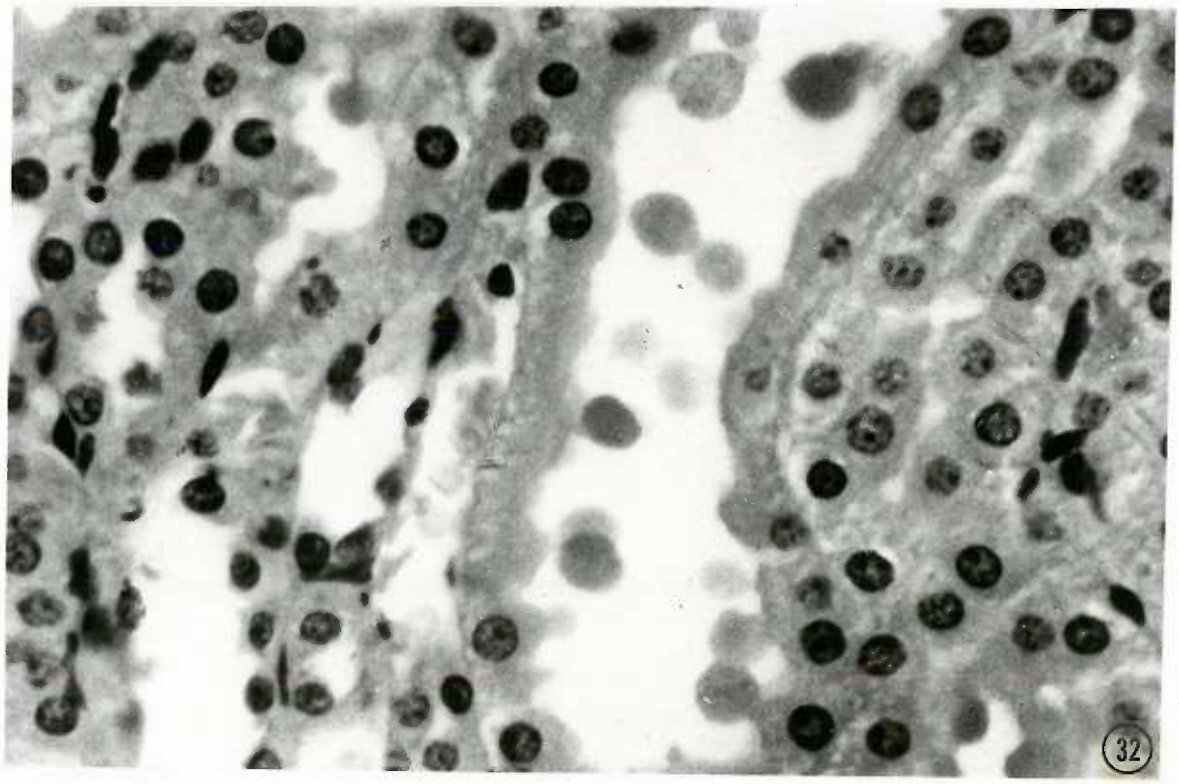

32

Figure 33.

Pars convoluta cell from a female rat given dieldrin plus CH_3HgCl for 84 days. Mitochondria are slightly swollen, but the architecture is otherwise similar to controls. X 20,500.

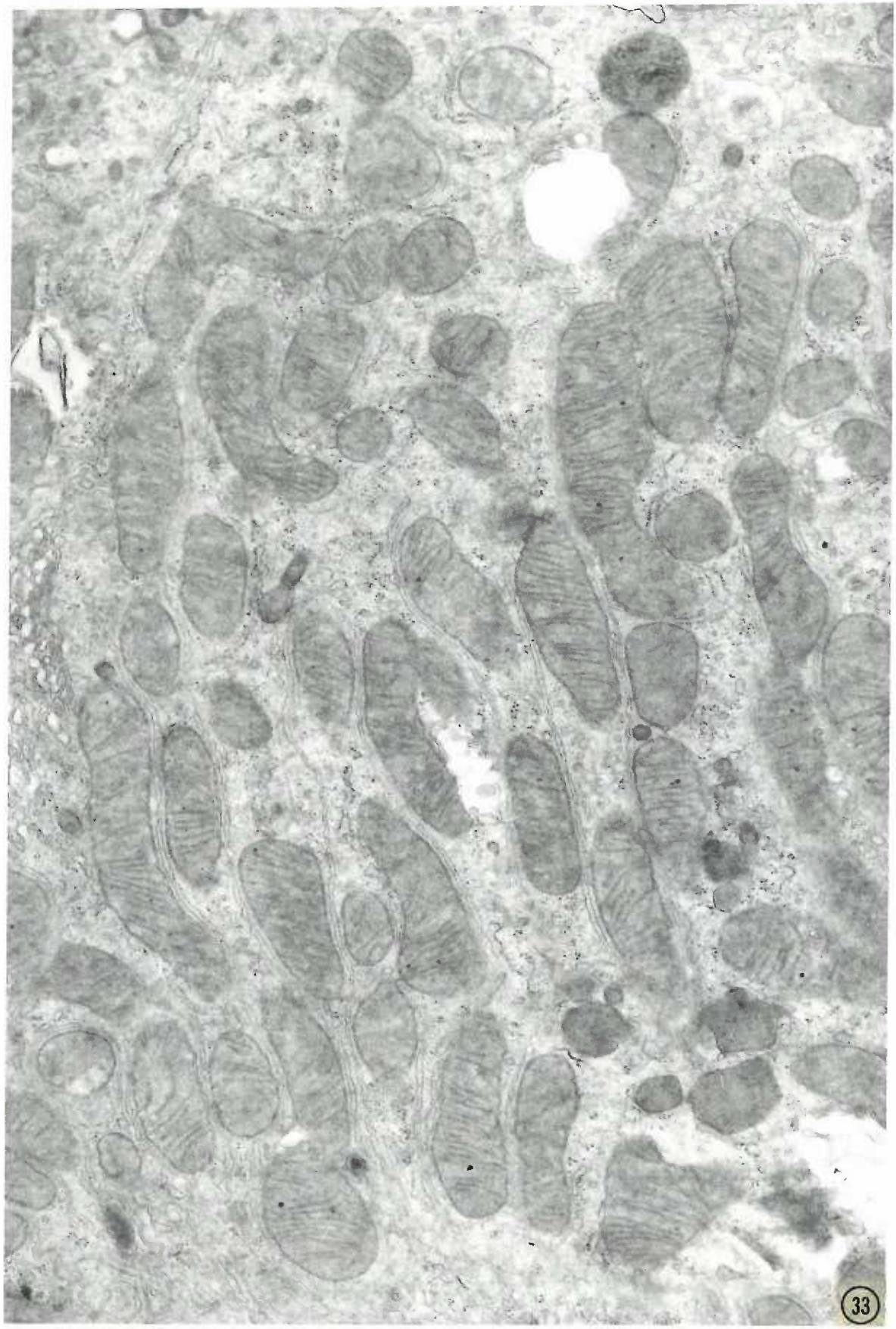

33

Figure 34.

Pars recta cell from a female fed dieldrin plus CH_3HgCl for 84 days. Note absence of dense membranous cytosomes and reduced amounts of SER in comparison to mercury-treated females. X 20,500.

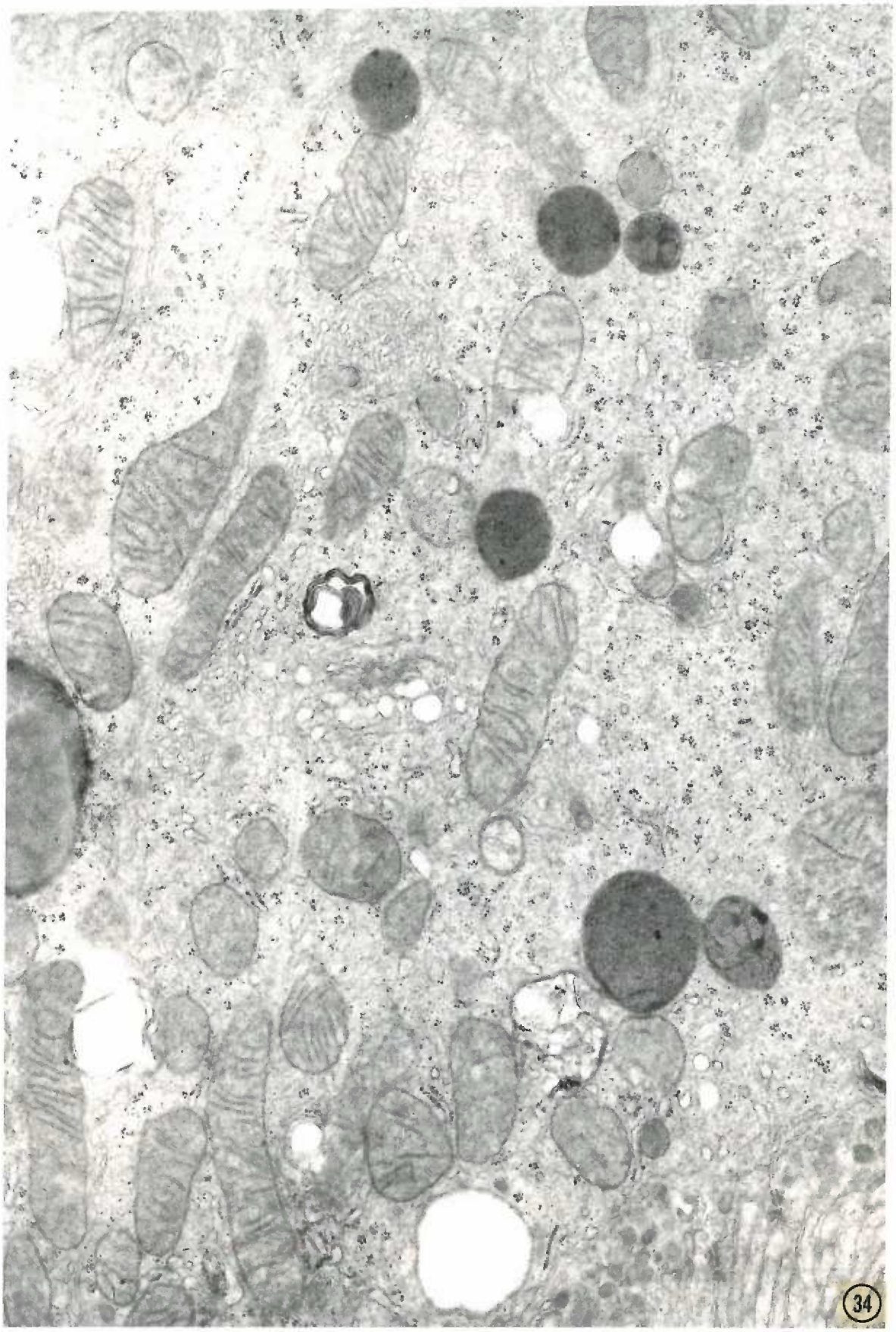

34

Figure 35.

A light micrograph of the inner renal cortex of a male rat treated with dieldrin plus CH_3HgCl for 84 days showing typical histologic appearance. The appearance is similar to those of control animals. X 220.

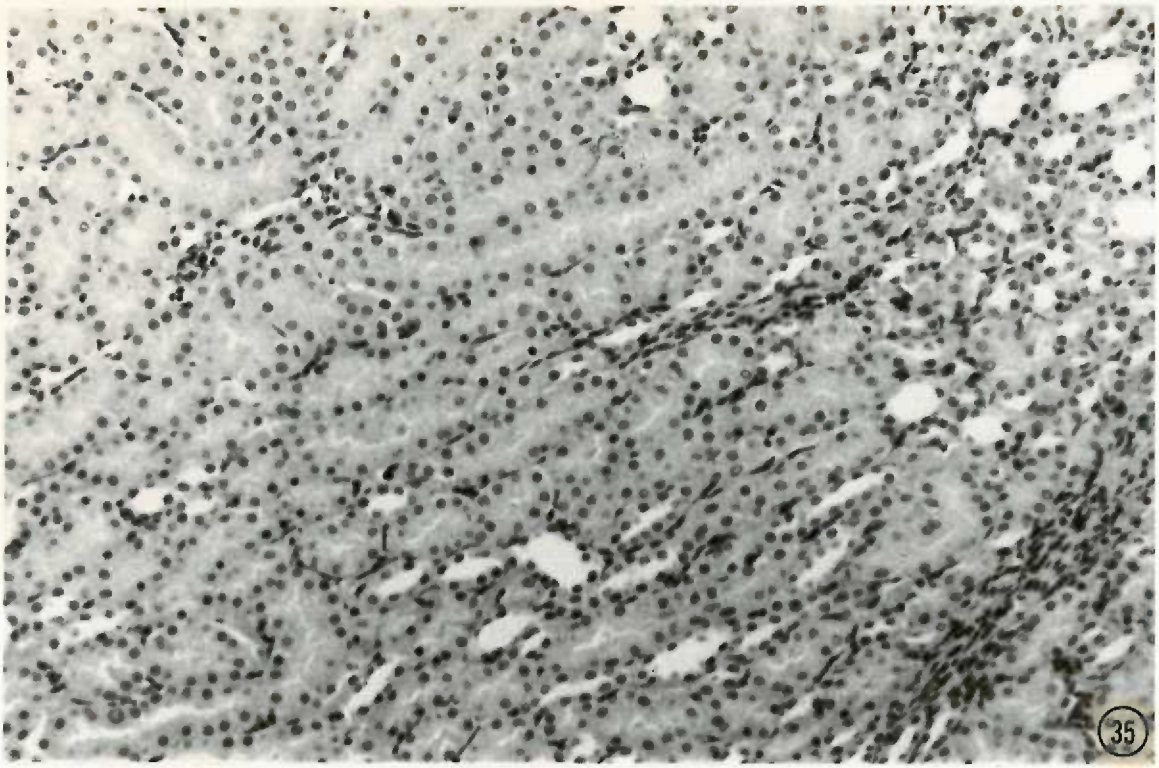

35

Figure 36.

Male rat exposed to dieldrin plus CH_3HgCl for 84 days showing typical appearance of a pars convoluta cell. X 18,500.

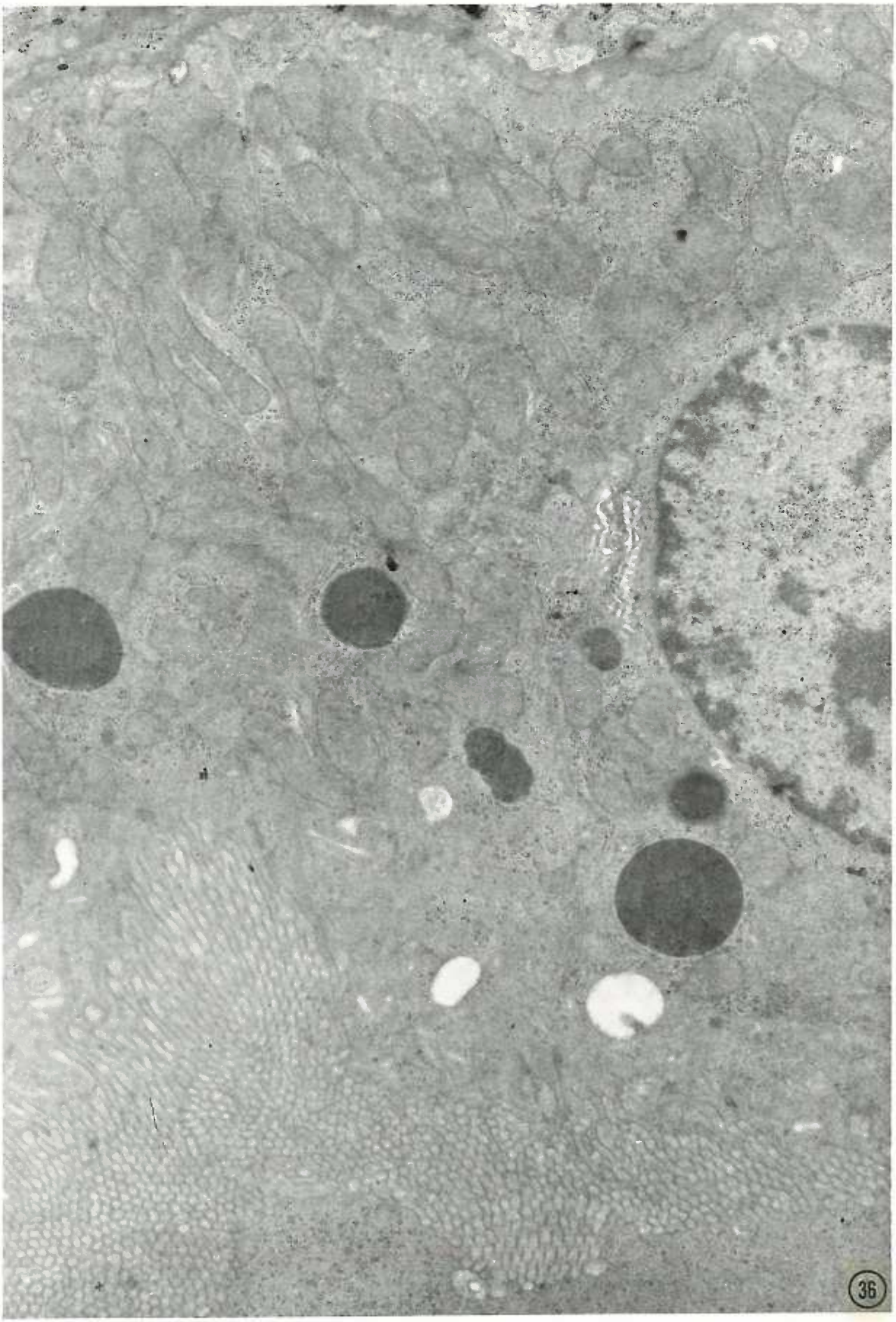
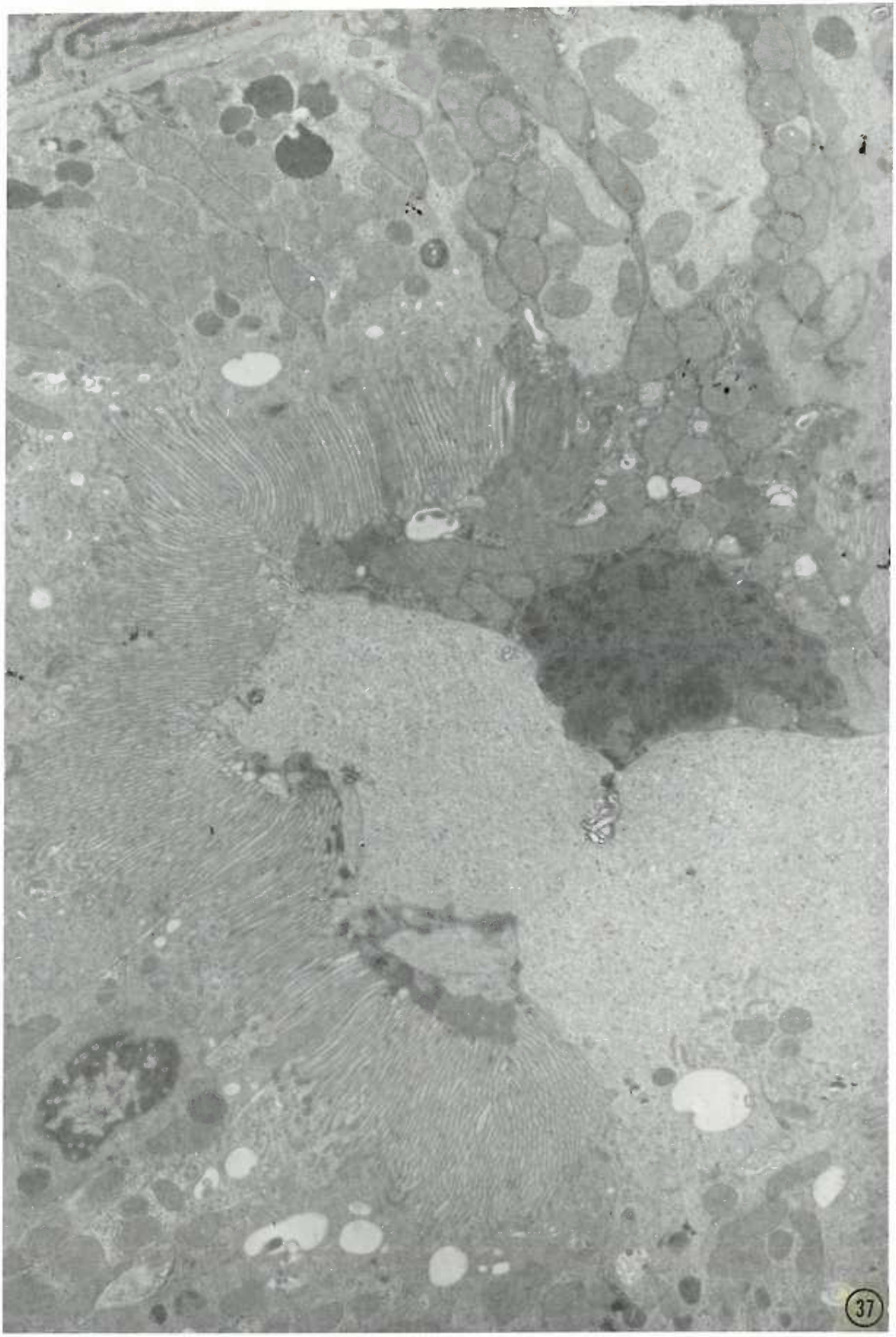

36

Figure 37.

Degenerating cell apparently in the process of being extruded into the lumen of a pars convoluta segment from a male rat administered dieldrin plus CH_3HgCl. X 15,400.


37