Electron Microscopic Observations on the Morphogenesis of Renal Cell Carcinoma Induced in Rat Kidney by Dimethylnitrosamine and N-(3,5-Dichlorophenyl)succinimide

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SUMMARY

Renal cell carcinoma was induced in rats by p.o. administration of dimethylnitrosamine, 500 ppm daily, followed by N-(3,5-dichlorophenyl)succinimide (NDPS), 5000 ppm daily. Fine structural changes of the proximal convoluted tubule cells were observed by sequential examinations of the kidney cortices at 3, 5, 12, and 24 weeks after drug administration. Early prominent structural changes of the cells induced by dimethylnitrosamine alone were the appearance of microspherules in nuclei and of numerous lamellar bodies on the membrane structure of the cells. With the addition of NDPS, the cells exhibited edematous cytoplasm that, in contrast to the relatively intact nuclear structure, contained numerous small vesicles and dark mitochondria, with markedly disarranged microvilli. After prolonged treatment with these drugs, some of the cells showed regenerating features, while others became necrotic. In the former case, large clear nuclei appeared with enlarged nuclei containing a large amount of granular components. Ribosomes in the cytoplasm also increased in number in accordance with nucleolar changes, and edema in cytoplasm and microvilli markedly decreased. However, a considerable number of vesicles still remained in some cells. Mitochondria decreased in number and showed pleomorphism and relatively high electron density. At 24 weeks, when clear cell carcinoma was induced, the cells in the cancer tissue exhibited a variety of features in their nuclei and cytoplasm. Some cells showed intact nuclear structure and dark cytoplasm containing a large number of vesicles; others had large round clear nuclei with enlarged nuclei and clear cytoplasm containing no vesicles. Among these cells were mixed populations of large clear cells, showing a structure similar to the cells at 12 weeks, i.e., to nodular hyperplastic cells. The starting point of malignant transformation seemed to be 1 week after treatment with NDPS (i.e., cells at 5 weeks) and, of the precancerous stage, at 12 weeks.

These results suggest that the proximal convoluted tubule cells previously damaged by dimethylnitrosamine treatment were marked for mutation and were transformed to cancer cells by additional treatment with NDPS in such a way as to disturb the permeability of the membrane system of the cell and to condense chromatin fibers.

INTRODUCTION

Spontaneous renal cell tumors may be induced in the rat through a diet containing a carcinogen. Administration of DMN p.o. has been shown to induce kidney tumors in about 60% of the experimental animals, about 15% of which were renal cell tumors (10).

In our laboratory, a new method (11) was recently developed to induce kidney tumors, in 100% of all experimental rats, of which 80% are renal cell tumors. This method involves p.o. administration of a diet containing DMN followed by NDPS. The renal cell tumor of such high incidence serves as a very useful model for tracing sequential development of this tumor. Histological (4, 10, 11, 14, 27) and ultrastructural (8, 12, 16) observations of renal cell tumors have been reported. Sequential changes in the proximal convoluted tubule cells of rats caused by DMN have been reported (9); however, the method used was single i.p. injection of DMN followed by a protein-free diet.

NDPS administered p.o. is known to cause chronic pyelonephritis (11). However, it is not known why rats that have been pretreated with DMN always develop kidney tumors when NDPS is administered and why the reverse administration procedure never induces the tumor. Although ultrastructural changes caused by NDPS have been reported (24), the relationship of these changes to malignant transformation has never been described on the ultrastructural level. As stated in a previous report (9), the proximal convoluted tubule cells, particularly the cells in the proximal part (21), were observed. It is the purpose of this experiment to observe what kinds of cellular changes occur in the course of carcinogenesis, particularly in the precancerous stage, and to see what kinds of cellular changes caused by NDPS have a causal relation to the malignant transformation of DMN-pretreated cells. Some of the results have been reported in another paper (23).

In previous reports on hyperplastic liver nodules (26) and hepatoma (22), we described remarkable changes in their nuclei and nucleoli. The present study focuses on the nuclear and nucleolar fine structure to see how the cytoplas-
mic change corresponds with the nuclear and nucleolar changes in the course of carcino genesis.

MATERIALS AND METHODS

Preparation of the diet containing DMN (Tokyo Kasei, Co., Tokyo, Japan) and NDPS (Sumitomo Chemical Co, Osaka, Japan) and the experimental plan are shown in Chart 1. Six- to 8-week-old male Wistar rats (CLEA Japan, Inc., Tokyo, Japan), weighing about 170 g, were used. The rats were fed for 2 weeks on a basal diet containing 500 ppm of DMN, for 2 weeks on a stock diet, for 8 weeks on a basal diet containing 5000 ppm of NDPS, and then for 12 weeks on a stock diet.

Rats were killed at 3 weeks (Group C), 5 weeks (Group D), 12 weeks (Group E), and 24 weeks (Group F). Two control groups consisted of the rats fed on the basal diet containing 5000 ppm of NDPS; they were killed at 4 weeks (Group A) or 6 weeks (Group B). Kidney cortices of both sides were excised while the animals were under anesthesia, and they were prepared for light microscopy, electron microscopy, histochemistry, and cytochemistry. For light microscopy, tissues were fixed in 10% formaldehyde solution, and paraffin-embedded sections were routinely stained with hematoxylin and eosin. For electron microscopy, tissues were fixed with 2.5% glutaraldehyde in cacodylate buffer, pH 7.2, at 4° for 1 hr; tissues were then dehydrated in graded ethanol and embedded in epoxy Epon resin. Thin sections made on LKB Ultrotome were doubly stained with saturated uranyl acetate and lead citrate. They were observed in a Hitachi Model HU-12 electron microscope. Electron micrographic observations revealed a wide range of variation in the tissue, a finding that correlates with the light microscopic observations of the paraffin sections and of the thick Epon sections stained with toluidine blue.

RESULTS

Group A

Animals were fed NDPS for 4 weeks; this was Control 1.

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0.05% Dimethyl nitrosamine (DMN)
0.5% N-(3, 5-dichlorophenyl) succinimide (NDPS)
Stock diet

Chart 1. Feeding schedules of the experimental groups.

Light Microscopy. As shown in Fig. 1, most of the proximal convoluted tubules were composed of dark cells that were smaller than normal. Their lumina were dilated but occluded by the apposition of extraordinarily developed brush borders.

Electron Microscopy. The proximal convoluted tubules showed various degrees of cell destruction; some consisted of completely destroyed cells while others were relatively intact. Microvilli were abnormally well developed at the luminal surface and in the intercellular spaces, resulting in the loss of their predilection for apical orientation and the occlusion of the lumina (Fig. 8). Individual microvilli were edematous and were varied in length, direction, and electron density. The nuclei of moderately damaged cells (Fig. 7) appeared clear; thick chromatin strands were dispersed in the nucleoplasm, condensed chromatin aggregates were lost, and the nucleoli had a small round compact form. The nuclei of seriously damaged cells (Fig. 8) appeared more electron dense than did those of moderately damaged cells; the nucleoplasm was filled with thick chromatin strands 150 to 250 Å wide (Fig. 9), a small or no mass of nucleolus remnant was found, and part of the nuclear envelope was ruptured. Mitochondria of the cells with relatively intact structure were large and edematous, with disarranged cristae, whereas the mitochondria of moderately or seriously damaged cells (Fig. 8) appeared dark and were decreased in number and size. In these edematous mitochondria the limiting membrane was destroyed, the matrix was filled with numerous small particles, and the cristae were destroyed (Fig. 10). The cytoplasmic matrix seemed either clear or rather edematous. Ribosomes were markedly decreased. Basal infoldings were poorly developed (Fig. 7), although in some cases they were complicatedly entangled with one another. These structural changes indicated edema of both nuclei and cytoplasm.

Group B

Animals were fed NDPS for 6 weeks; this was Control 2.

Light Microscopy. As Fig. 2 illustrates, the tissue showed chronic active pyelonephritis. The proximal convoluted tubules were composed mainly of clear cells; their lumina were not so dilated or occluded with the apposition of brush borders as in the former experiment (Group A). Distal convoluted tubules contained cell debris in their lumina. Glomeruli seemed intact.

Electron Microscopy. Most of the proximal convoluted tubule cells appeared to have intact cell structures, although some continued to show destroyed features. In the nucleus of such an intact cell (Figs. 11 and 12), the nucleoplasm increased its electron density, and many large condensed chromatin aggregates appeared. The nucleolus was enlarged and surrounded by large nucleolus-associated chromatin. Microspherules were never seen. These nuclear and nucleolar features indicated that the nucleus was in the preprophase. Compatible with these nuclear structures, the cytoplasmic matrices increased in electron density, and polysomes and RER were increased. Mitochondria were distributed throughout the cytoplasm without particular localization. Much variation in their size and shape was
noticed (Figs. 11 and 12). Their matrices had such high electron density that well-developed cristae were scarcely visible. Basal infoldings were well developed and complicatedly entangled with each other (Fig. 11). These nuclear and cytoplasmic structures indicated normal or somewhat regenerating features, suggesting restoration from the damage caused by the drug.

**Group C**

Animals were fed DMN for 2 weeks and then stock diet for 1 week; tissue was observed at 3 weeks.

**Light Microscopy.** Fig. 3 shows that proximal convoluted tubules were composed of practically normal cells, except for enlarged nuclei and a few vesicles in some cells. Interstitium was edematous and slightly infiltrated with interstitial cells.

**Electron Microscopy.** The cells of the proximal convoluted tubule exhibited a wide range of variation in their fine structures. Most of the nuclei (Fig. 13) were large in size, with some large condensed chromatin aggregates. Nucleoli were also enlarged, and they assumed a slightly compact form. Many microspherules were always encountered in their fibrillar components (Fig. 14). In some nuclei, however, chromatins were completely dispersed in the nucleoplasm, giving the appearance of clear nuclei. Such nuclei usually contained a lamellar body in their nucleoplasm. Microvilli, apical vesicles, and basal infoldings appeared intact, but intracytoplasmic dense bodies (lipid and probably protein droplets) were scarcely increased. Mitochondria were usually enlarged and increased in number (Fig. 13); a large one at the bottom of the cell in Fig. 15 showed well-developed cristae of tubular form, indicating high enzymatic activity. Numerous lamellar bodies appeared on the limiting membrane of cell organelles such as mitochondria and RER. These also appeared on the nuclear envelope and even in the nucleoplasm (Fig. 16).

**Group D**

Animals were fed DMN for 2 weeks and subsequently were fed NDPS for 1 week after an interval of stock diet for 2 weeks. Tissue was observed at 5 weeks. The structural changes induced in this experiment may be regarded as the acute toxic effect of NDPS.

**Light Microscopy.** As Fig. 4 reveals, the proximal convoluted tubules were composed of dark cells, and their lumina were slightly dilated, being occluded by brush borders. Distal convolutions were composed of clear cells, and their lumina were occluded by cell debris. Glomeruli were intact. Interstitium was slightly infiltrated with interstitial cells.

**Electron Microscopy.** As in Group C, the proximal convoluted tubules did not all show the same structural changes. In a seriously damaged cell (Fig. 17), well-developed microvilli were edematous and they varied in number, size, and length. The cytoplasm (Figs. 17 and 19) contained numerous vesicles of various sizes and shapes. Some vesicles were united with one another by attachment to the plasma membrane or by direct communication with the uniniferous lumen. Some of the vesicles seemed to originate from the RER, since ribosomes arranged in a row on their outer surface were noted (Fig. 19). Small round mitochondria were scattered in the dark cytoplasm. An electron-dense substance filled the mitochondrial matrices and intracisternal spaces and obscured the intramitochondrial structure. Ribosomes of single and polysomal types were decreased in number. Many small lipid droplets were frequently encountered, whereas lamellar bodies were markedly decreased. Usually, basal infoldings were poorly developed. Basal lamina appeared to have normal thickness. In contrast to these cytoplasmic changes, the nuclear structure remained intact (Fig. 17), with electron-dense nucleoplasm and a moderate number of condensed chromatin aggregates. Most of the nucleoli, assuming compact form, contained a number of microspherules at the fibrillar components (Fig. 18). These microspherules were more numerous and more prominent than those of the nucleoli that were treated with DMN alone (Group C).

**Group E**

Animals were fed NDPS for 7 weeks in addition to the feeding schedule of the preceding group (Group D). Tissue was observed at 12 weeks.

**Light Microscopy.** As shown in Fig. 5, the proximal convoluted tubule cells were hyperplastic, and their lumina were occluded with a small amount of cell debris. Nucleoli and nucleoli were enlarged, and intertubular spaces were infiltrated with a few interstitial cells.

**Electron Microscopy.** As shown in the experimental group fed NDPS for 6 weeks (Group B), the epithelial cells of the individual tubule usually displayed repairing or even regenerating features from the damage by the drug, although some cells maintained structural damage similar to that of the cells observed at 5 weeks (Group D). Microvilli were usually well developed (Fig. 20), appearing even in the intercellular spaces. The microvilli and cytoplasmic matrices appeared intact; edemas were remarkably decreased. Some cells still maintained numerous intracytoplasmic vesicles (Fig. 20), although most of the cells contained only a few vesicles. Mitochondria showed considerable variation in size and shape (Fig. 20). They were generally decreased in number and scattered throughout the cytoplasm. Although their matrices and intracisternal spaces were filled with electron-dense substance, well-developed cristae were observed to be well preserved (Fig. 22). Pleomorphism, such as very small mitochondria and elongation and attenuation of the midregion of large mitochondria, was frequently encountered, suggesting regeneration of mitochondria (25). Free ribosomes were increased in number, most of which were polysomal in type. A moderate amount of small lipid droplets and only a few lamellar bodies on the limiting membrane of cell organelles still remained. Development of basal infoldings was very poor.

Consistent with these cytoplasmic changes, the nuclei displayed regenerating features. They were large and their nucleoplasm was relatively electron dense. Small condensed chromatin aggregates were attached to the inner layer of the nuclear envelope or were scattered in the nucleoplasm (Fig. 20). The nucleolus (Fig. 21) assumed a...
compact form, in which numerous granular components in the central region and a few fibrillar components at the peripheral region were noted. Small condensed chromatin was associated with a part of the nucleolus. No microspheres were found. Basal lamina increased in thickness in some parts of the tissue (Fig. 20).

**Group F**

Animals were fed stock diet for 12 weeks after treatment with DMN and NDPS; renal adenocarcinoma was established.

**Light Microscopy.** As shown in Fig. 6, the tissue was composed of clear cells with large round nuclei and nucleoli and clear cytoplasm. The carcinoma showed considerable variation in cytological patterns of growth. Mitotic figures were frequently encountered among these cells. Lymphocyte infiltration appeared among tumor cells and in intertubular spaces.

**Electron Microscopy.** The tissue was composed of a mixed population of cells showing cell organelles with various kinds of features, and well-developed interstitium. The cancer cells (Fig. 23) were roughly divided into 2 types. One type was a cell containing numerous vesicles of irregular shape in the dark cytoplasm; the other type contained no such vesicles. It was impossible to determine whether these cells originated, either in toto or partially, from the proximal convoluted tubule cells, since they had microvilli to a greater or lesser extent.

Cells of the 1st type were dark cells containing numerous vesicles of various sizes and shapes in the cytoplasm (Figs. 23 and 24). This type of cell had a structure similar to that of the cell examined at 12 weeks (Fig. 20) and must have persisted from the former stage (Group E). The large, irregularly shaped nuclei contained numerous small, condensed chromatin fibers that were scattered in the nucleoplasm or attached to the nuclear envelope. The large and irregularly shaped nucleoli contained a large amount of granular components (Fig. 24). Cytoplasm contained a number of vesicles of irregular shape, containing amorphous substance. Numerous polysomes filled the dark cytoplasm. Mitochondria were small in number and size, showing a clear appearance. Many small lipid droplets were seen in some cells (Fig. 23).

Cells belonging to the 2nd type mentioned above were commonly seen (Fig. 25). The nuclei in this type was large and either oval or irregular, and the nucleoplasm appeared relatively clear. Numerous small, condensed chromatin fibers were free in the nucleoplasm or were attached to the inner layer of the nuclear envelope, indicating the presence of numerous nuclear pores. A considerably large nucleolus was composed of well-separated nucleolonomas and a small amount of associated chromatin fibers. Small intranucleolar chromatin fibers were always situated in the clear spaces of the nucleolus. Such a nucleolar structure had not previously been seen in the course of this experiment, which indicated good differentiation of the nucleolus. In the cytoplasm, no vesicles could be seen. The RER and Golgi complex were well developed, and a few free ribosomes were scattered throughout the cytoplasm. Mitochondria were decreased in size, round or elongated, and distributed throughout the cytoplasm; however, no electron-dense substance was seen in their matrices and intracisternal spaces. Lamellar bodies on the limiting membrane were rarely encountered.

Other parts of the cancer tissue were composed of large, clear cells that were usually found in hyperplastic nodules. The cells (Fig. 26) were very similar to the cells of the 2nd type in the cancer tissue. The nuclei were large and round, the chromatin fibers were dispersed in the nucleoplasm, which showed a clear appearance, and many small condensed chromatin fibers were free in the nucleoplasm or attached to the inner layer of the nuclear envelope. However, unlike cells of the 2nd type, these cells had large and irregular nucleoli (Fig. 27) that were composed of a large amount of granular components roughly in the central area, fibrillar components at the peripheral area, and a small amount of associated chromatins. In such cells, free polysomes were markedly increased in number and were scattered throughout the cytoplasm (Fig. 26). Mitochondria that were of orthodox form (19) were decreased in number and size. These cells were believed to be hyperplastic nodular cells or precancerous cells and to have high activities of DNA and RNA synthesis (26).

The development of the basal infoldings was very poor or completely lacking (Figs. 23, 24, and 26). Basal lamina varied in thickness and reached a few nm in some places. As reported previously (8), microvilli were developed in the intercellular spaces between tight junctions (Fig. 28), or they covered the entire cell surface except for the basal region (Fig. 29) or were included in the cytoplasm at the basal region (Fig. 30).

**DISCUSSION**

To observe tissue in the course of carcinogenesis, in which various degrees of cell differentiation and of cell destruction are intermixed, it is essential to observe not only intact cells but also necrotic ones, and to investigate changes not linked to the process of malignant transformation.

NDPS causes chronic active pyelonephritis (11), but it is not thoroughly understood how the drug destroys the proximal convoluted tubule cells. As shown in the control experiment, tubule cells exhibited considerable variation in structure, ranging from necrotic to intact. In an early stage of the treatment, NDPS (24) clearly induced edema of the cytoplasm, enlargement of apical vesicles, and cisternae of RER in the proximal convoluted tubule cells. These events indicate the destruction of and/or the causation of abnormally high permeability of the membrane system, such as the plasma membrane and limiting membranes of the vesicles. In the seriously damaged tubule, edematous epithelial cells became necrotic and appeared in the unirhinous tubules as casts. Since the glomerulus was intact, the filtrate containing NDPS in the tubule must easily attack the intertubular spaces directly or indirectly through the disintegrated epithelial cells, resulting in the induction of edema of the interstitium and interstitial cell infiltration.

Since many of the tubules were repaired from the damage after a 6-week exposure (Group B), the cells are presumed to be capable of metabolizing NDPS to a nontoxic substance after prolonged treatment.
Maximum necrobiotic changes occurred after treatment for 4 weeks (Group A); in these cells, some of the nuclei showed prominent structural changes with serious edema of the cytoplasm. Chromatins never showed the clumping usually seen in karyorrhexis but were dispersed diffusely in the nucleoplasm, forming thick strands 250 Å wide (Fig. 9). Since the width was double or more that of normal chromatin strands of 40 to 150 Å width (13), they seem to be formed through the condensation of more than 2 chromatin fibers.

These necrobiotic changes show the effects of NDPS on the cells, particularly on the chromatins, and presumably have no relation to carcinogenesis. However, these changes must be linked to carcinogenesis when NDPS attacks the DMN-pretreated cells.

Since only the procedure of using DMN followed by NDPS induced a 100% incidence of renal tumors, the DMN-pretreated cells must certainly be transformable to cancer cells at any point after the cells are damaged by NDPS to some degree. The critical time of premalignancy is presumed to be 1 week after NDPS treatment (Group D), since the cells are designated for carcinogenesis with NDPS treatment. This significant structural changes that appeared in the tubule cells at this time were abnormally developed edematous microvilli and the appearance of numerous vesicles in the edematous cytoplasm. These changes indicate abnormal permeability of the membrane system induced by NDPS. There must be a disturbance of cation transport in the cells; however, the nuclei of such cells showed an intact chromatin structure. Some invisible structural changes in the genes must have been produced in this early experimental stage, because the nucleoli in such cells always contained a number of microspheres, indicating destruction of RNA polymerase (1).

After prolonged treatment with NDPS, both the DMN-pretreated and nontreated cells recovered from the damage. Edema and vesicles of the cytoplasm, which were found in the cells in an early stage, were observed. However, the repaired features were considerably different from each other. The DMN-pretreated cell nuclei (Fig. 20) showed structures similar to those of hyperplastic nodular cells (26), whereas the nontreated cell nuclei (Fig. 11) showed normal structures of this cell or, rather, cell division. These differences are likely to be attributable to whether the nuclei were previously damaged by DMN at an early stage. None of the tissues at 12 weeks appeared cancerous in paraffin section or in Epon thick sections. On the other hand, the tissue at 24 weeks (Fig. 6) was obviously renal adenocarcinoma as observed by light microscopy. We wonder if an important transforming period to carcinoma between 12 and 24 weeks was not observed; it remains to be clarified in the future.

In an electron microscopic observation, however, cells appearing very similar in structure to the cells at 12 weeks were always found among the cancer cells or in a part of the cancer tissue (Fig. 26), particularly in the area showing hyperplastic nodules. Although we did not observe transforming tissue between 12 and 24 weeks, it is untenable that 12-week cells are not precancerous (possessing an ability to transform into malignant cells).

The nuclei in the cancer cells showed considerable variation in their fine structures, as was previously reported in the hepatic cell tumor (22). This variation seems to be attributable to differences in differentiation, maturation, and metabolic activities of individual cells.

As shown in Figs. 20 and 26, the large clear nuclei with enlarged irregularly shaped nucleoli usually appeared in the hyperplastic nodular cells and have high activity of DNA and RNA synthesis (26). Since these nuclear and nucleolar changes are usually encountered and reproducible in the proximal convoluted tubule cells in the course of carcinogenesis, they are believed to have an intimate relationship to malignant transformation, although some authors (2, 9) have suggested no relation to neoplastic development.

The appearance of the large clear nucleus must be due to some factors that have induced the twisted and condensed chromatin fibers to extend and disperse into the nucleoplasm. One of the factors expected (5) is a serious damage of ligand macromolecules to DNA, such as histone; another is an increase of counterligands that function as antagonists to DNA-histone interaction, such as residual proteins, RNA, phosphoproteins, and phospholipids. With either or both of these factors, DNA may easily disperse in the nucleoplasm and function as template in DNA replication. DMN is believed to affect DNA (3), RNA (20), and protein (1) and to accumulate lipid (18) in liver parenchymal cells. In addition to these DMN effects, when NDPS acts on the nucleus there is condensation of the chromatin and an imbalance of cation in the nucleoplasm; the mutation of genes may be surely induced.

The clear nucleus that appeared in the cells after treatment with DMN for 2 weeks (Group C) seems to be formed by dispersion of chromatin fibers, but it is different from the clear nucleus that appeared in a later stage (Groups E and F). Such dispersion of chromatin fibers seems to indicate disappearance of DNA caused by the metabolic disturbance of lipoprotein, since such nuclei usually contained lamellar bodies in the nucleoplasm. Such lamellar bodies in the nucleus never appeared in the latest experimental stage; therefore the nucleus is looked to for the earliest morphological manifestation of DMN intoxication, as previously reported (9, 27).

Microspheres in the nucleolus, which appeared in the cells treated with DMN for 2 weeks (Group C), indicate the destruction of RNA polymerase in the nucleolus, as previously reported (1). They cannot be regarded as a sign of cancer, since they are also induced by noncarcinogens such as actinomycin D and other drugs (1). They increased considerably in number after treatment with NDPS for 1 week (Group D), and they completely disappeared in the consecutive treatment of 8 weeks (Group E); thus they seem to be attributable to the toxic effect of DMN.

Numerous vesicles in the cytoplasm, which are believed to originate from apical vesicles and RER, seem to be closely related to the toxic effects of NDPS. However, the cells containing such vesicles are not always destroyed and can be restored to normal cells. Although RER may be dilated by NDPS at an early stage, it may be possible that the drug-metabolizing enzyme in RER converts the drug to a nontoxic substance. In the cells treated with DMN and NDPS for long time periods (Group B) and in cancer cells, an imbalance was found between nuclear and cytoplasmic structures. The cytoplasm was filled with numerous large
vesicles, indicating serious damage, while the nucleus displayed features characteristic of high metabolic activity (Figs. 23 and 25). This indicates that the cells are repairing from the toxic damage, probably, by the drug-metabolizing enzyme in numerous vesicles.

Enlarged mitochondria with elaborately developed cristae appeared in the cells at an early experimental stage (Group C); they are believed to indicate intoxication induced by DMN, as in the case of Cuprizon intoxication in the hepatic mitochondria (25). They can be restored to a normal or small size by removing the drug from the diet. Their pleomorphism in a later experimental stage (Group E) is suggestive of recovery from their toxic damage. After combined treatment with DMN and NDPS, mitochondria decreased in number and size and they contained electron-dense substance in their matrices. These mitochondrial changes are believed to be caused by NDPS, since the dark mitochondria appeared in the control experiment with NDPS alone and were absent in the cells treated with DMN alone. In cancer cells, dark mitochondria in orthodox form appeared only in the cells containing numerous vesicles in their cytoplasm, and they disappeared in the cells that had only a few small vesicles (19). It is not clear whether such dark mitochondria have an intimate relationship to cancer and whether the mitochondrial deficiency is a phenomenon of cancer only or a consequence of the effect of NDPS.

The lamellam bodies that appeared in the cells in an early experimental stage with DMN treatment are consistent with those that appeared in mouse kidney or rat lung, as previously reported (6, 17). Since they are believed to store and/or synthesize essential components of surface-active lipoprotein (16), their numerous appearances on the membrane structure are suggestive of a restoration of the membrane from the damage caused by DMN. However, they are presumed to have no relation to cancer, since they disappeared or markedly decreased in the later experimental stage.

Although abnormally developed microvilli are one of the characteristic features of renal cell carcinoma (8, 16), it is not clear whether they have some relationship with carcinoma, because a high incidence of renal tumors is induced in the absence of acute structural damage in the cells (15), and no renal tumor is induced in the presence of severe structural damage as in the case of NDPS and other drugs (7).

REFERENCES

Figs. 1 to 6. Light micrographs of kidney cortices. Figs. 1 to 5. Epon-embedded thick sections stained with toluidine blue. Fig. 6. Paraffin section stained with H & E. Figs. 7 to 30. Electron micrographs of ultrathin sections stained with uranyl acetate and lead citrate.

Fig. 1. Group A (Control 1). Tissue from animals treated with NDPS for 4 weeks. Proximal convoluted tubule (P) in the corticomedullar zone is constituted with dark epithelial cells, with dilated urinary lumina (L). I, edematous interstitium. × 100.

Fig. 2. Group B (Control 2). The tissue, from animals treated with NDPS for 6 weeks, shows chronic active pyelonephritis. G, intact glomerulus; P, proximal convoluted tubule; D, distal part of nephron containing cell debris. × 100.

Fig. 3. Group C. Tissue from animals treated with DMN for 2 weeks followed by stock diet for 1 week. Epithelial cells in the proximal convoluted tubules (P) show practically normal structures, except for large nuclei and small vesicles (arrow). I, infiltrated interstitium. × 400.

Fig. 4. Group D. Tissue from animals treated with DMN for 2 weeks, followed by stock diet for 2 weeks and NDPS for 1 week. Proximal convoluted tubule (P) shows dark appearance, in contrast with distal convoluted (D) showing clear appearance and containing cell debris in their lumina. G, intact glomerulus; I, interstitium. × 100.

Fig. 5. Group E. Tissue from animals treated with DMN for 2 weeks, followed by stock diet for 2 weeks and NDPS for 8 weeks. Cortical area shows hypertrophy of proximal convoluted tubule. Most of the epithelial cells (P) show practically normal configuration, with clear cytoplasm and large nuclei (N). Their lumina still contain cell debris (arrow). I, poorly developed interstitium. × 400.

Fig. 6. Group F. Established clear cell carcinoma after treatment with DMN for 2 weeks, followed by stock diet for 2 weeks, NDPS for 8 weeks, and stock diet for 12 weeks. The tissue is composed of a clear cell population and a relatively well-developed interstitium. × 100.

Fig. 7. Group A (Control 1). Epithelial cells in the proximal convoluted tubule are seriously damaged, showing edema in nuclei and cytoplasm. NU, nucleus; M, mitochondria; V, vesicle; BL, basal lamina. × 6,350.

Fig. 8. Group A (Control 1). A seriously damaged cell. The uniniferous tubule is occluded with extraordinarily developed microvilli (MV) of irregular shape and length. N, destroyed nucleus; M, edematous mitochondria. × 6,350.

Fig. 9. Enlarged micrograph of the nucleus in Fig. 8. Chromatin fibers are from 150 (small arrow) to 250 Å (large arrow) wide. × 85,000.

Fig. 10. Enlarged micrograph of edematous dark mitochondria. The matrix is filled with numerous small particles; the limiting membrane (LM) and cristae (C) are destroyed (arrow). × 110,000.

Fig. 11. Group B (Control 2). Epithelial cell showing intact structures in nuclei and cytoplasm. BI, basal infolding. × 7,000.

Fig. 12. Enlarged micrograph of the cell in Fig. 11. C, condensed chromatin; NU, nucleus; NAC, nucleolus-associated chromatin; G, granular component; F, fibrillar component; R, polysome; M, mitochondria. × 28,000.

Fig. 13. Proximal convoluted tubule cells in Group C. N, nucleus; C, condensed chromatin; NU, nucleus; M, mitochondria; BI, basal infolding; MV, microvilli; V, apical vesicle; D, dense body; arrows, lamellar bodies. × 9,000.

Fig. 14. Higher magnification of the nucleolus in a cell in Group C. S, microsphenum; F, fibrillar component; G, granular component; NAC, nucleolus-associated chromatin. × 120,000.

Fig. 15. Large mitochondria at the cell bottom of a cell in Group C. It is clearly visible that its cristae take tubular form, from their round or oval shape in transverse section (arrows). BI, basal infolding. × 60,000.

Fig. 16. Enlarged micrograph of a cell in Group C. Lamellar bodies (L) are found in the nucleoplasm, on the outer layer of the nuclear envelope (arrow), and on the limiting membrane of the mitochondria (M). × 90,000.

Fig. 17. Seriously damaged cells in Group D. In contrast to marked damage in the cytoplasm, the nucleus (N) shows a relatively intact structure. MV, edematous microvilli; V, intracytoplasmic vesicle; M, mitochondria. × 8,750.

Fig. 18. Enlarged micrograph of the nucleus of the kidney that is commonly encountered in Group D. S, microsphenum; F, fibrillar component; G, granular component. × 50,000.

Fig. 19. Enlarged micrograph of a part of the cytoplasm of the cell in Fig. 17. MV, edematous microvilli; UV, united vesicle; V, small vesicle; MY, myelin figure; R, ribosomes arranged in a row on the limiting membrane of the united vesicle. × 25,000.

Fig. 20. Proximal convoluted tubule cells in Group E. In cell 1, many mitochondria are concentrated in the apical region; in cell 2, many vesicles (V) and mitochondria showing pleomorphism are found in the cytoplasm; and in cell 3, only a few vesicles and a small number of mitochondria are scattered in the cytoplasm. C, small condensed chromatin aggregates; NU, nucleus; BI, basal infolding; BL, basal lamina. × 8,400.

Fig. 21. Enlarged micrograph of the compact nucleolus of Cell 2 in Fig. 20. F, fibrillar component; G, granular component; NAC, nucleolus-associated chromatin. × 50,000.

Fig. 22. Higher magnification of the dark mitochondria usually found in Group E. Limiting membrane and cristae are well preserved. C, cristae; R, polysome. × 90,000.

Fig. 23. Two types of cancer cell (Group F). Cell 1, belonging to type 1, contains numerous intracytoplasmic vesicles (V), and small lipid droplets (L). Cell 2, belonging to type 2, contains only few vesicles in the cytoplasm. Cell 3, containing a moderate amount of small vesicles is regarded as an intermediate type between them. N, nucleus; I, interstitium. × 6,000.

Fig. 24. A cell belonging to type 1. Numerous vesicles (V) containing amorphous substance are varied in size and shape. C, small condensed chromatin aggregate; NU, nucleus; L, small lipid droplet. × 8,750.

Fig. 25. A cell belonging to type 2. N, large round clear nucleus; C, small condensed chromatin; NU, well-differentiated nucleolus; M, mitochondria; G, Golgi complex; large arrows, nuclear pores; small arrows, intranucleolar chromatin. × 12,500.

Fig. 26. Large clear cells in a part of the cancer tissue. N, large round clear nucleus; NU, nucleus; C, small condensed chromatin; R, polysome; M, mitochondria; BL, basal lamina. × 8,750.

Fig. 27. Higher magnification of the irregularly shaped nucleolus in Fig. 26. G, granular component; F, fibrillar component; NAC, nucleolus-associated chromatin. × 40,000.

Fig. 28. Development of microvilli (MV) at the intercellular spaces. × 18,500.

Fig. 29. Microvilli (MV) covering all the cell surface except for the basal region and tight junction. × 7,500.

Fig. 30. Microvilli (MV) developed in the cytoplasm of the basal region of the cell. × 10,000.
Morphogenesis of Renal Cell Carcinoma

[Image: Two electron micrographs showing renal cell carcinoma. The images are labeled 12 and 13.]

- The first image (12) shows a renal cell with prominent nuclei (N), cytoplasm (C), and associated organelles like the endoplasmic reticulum (R ER).
- The second image (13) highlights the cellular structure with a focus on the nuclei (NU) and other cell components such as mitochondria (M) and vesicles (V).

These images provide insights into the cellular architecture and morphological characteristics of renal cell carcinoma.
Morphogenesis of Renal Cell Carcinoma

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Electron Microscopic Observations on the Morphogenesis of Renal Cell Carcinoma Induced in Rat Kidney by Dimethylnitrosamine and N-(3,5-Dichlorophenyl)succinimide

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