Ultrastructural Study of the Development of Interstitial Lesions Leading to Mesenchymal Neoplasia Induced in the Rat Renal Cortex by DimethylNitrosamine

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SUMMARY

Within 24 hr following the administration of a single i.p. injection of dimethylNitrosamine in doses that result in up to 100% incidence of renal mesenchymal tumors, intracellular damage is seen with the electron microscope in interstitial cells with fibroblast morphology in the vicinity of glomeruli. Within several days of treatment, some periglomerular cortical fibrocytes and capillary endothelial cells are stimulated into division. An interstitial mononuclear inflammatory reaction, in which the macrophage is the dominant cell form, reaches a peak of intensity by 7 days. From 3 weeks on, a few glomerulus-associated hypercellular foci persist. The dominant cell type at this stage is the lymphocyte, although by 8 weeks plasma cells are also numerous. Abnormal fibroblast-like cells are noted in very small but increasing numbers within persisting lesions from 3 weeks after drug administration. By 12 weeks, there is a marked reduction of immunological cells, and persisting lesions consist predominantly of aggregations of fibroblast-like cells ultrastructurally identical with spindle or stellate cells of well-developed tumors. By 20 weeks, the proliferating lesions display the broad spectrum of vascular differentiation characteristic of these tumors. The findings are discussed with respect to the correlation between the target cell of acute injury and the ultimate development of neoplasm and the significance of abnormal cell forms present in early, persisting lesions.

INTRODUCTION

DMN¹ administered as a single i.p. injection to rats that have been subjected to certain dietary conditioning induces up to 100% incidence of renal mesenchymal tumors (7, 12, 23). The evolution of these neoplasms has been traced at the light microscope level from 24 hr after the carcinogenic dose up to 25 weeks, when macroscopic tumors may be visible (8). The study demonstrated that a few small hypercellular foci began to develop in the interstitial space around the glomerular hilus at about 4 days, and this seemed to be associated in part with the presence of dividing cells in this location from 2 days onwards. The interstitial reaction, a mononuclear inflammatory response, reached a peak at 7 to 14 days, and thereafter these foci progressively diminished in number over the ensuing weeks. In rats destined to develop renal mesenchymal tumors, chronic interstitial lesions persisted, consisting predominantly of lymphocytes and plasma cells, which merged into microscopic but unequivocal tumor cell aggregations by 16 weeks. Sporadic cells of large size, with clear nuclei and prominent nucleoli, found in early chronic lesions were histologically similar to mesenchymal tumor cells, suggesting that they may have been early components of neoplastic process. The sudden appearance of aggregations of tumor cells at 12 to 16 weeks seemed to be associated with a relative decrease in the number of lymphoid and plasma cells in persisting lesions. In this paper, we describe the ultrastructural character of this interstitial reaction, with the intention of determining the nature of the acute cellular damage and its significance with respect to ultimate tumor formation and identifying the constituent cell types of persisting lesions.

MATERIALS AND METHODS

The preparation of DMN and rats, the experimental plan, and the preparation of material for electron microscopy are all described in detail in other papers (7—9). In brief, male Wistar rats of the Porton strain, weighing 90 to 110 g, were given i.p. injections of 30, 50, or 60 mg/kg DMN, after 1 week of protein depletion (16) in the case of the 2 higher doses. Rats were killed, and their kidneys were prepared for electron microscopy at 1, 2, 3, 4, and 7 days and 2, 3, 4, 6, 8, 12, 16, and 20 weeks. Fixation was commenced by intravascular renal perfusion (5) with 2% glutaraldehyde in Veronal salt buffer (14) or a mixture containing 3% glutaraldehyde and 1% osmium tetroxide in collidine buffer (25). Whole, sagittal slices of kidneys, 1 to 2 mm thick, were fixed for a further period, dehydrated, and embedded in Epon. Two-μm sections of whole kidney, cut with a Porter-Blum MT-1 ultramicrotome (4, 15), were examined by light microscopy for fields of interest, and the relevant areas on the Epon blocks were isolated and sectioned with an LKB Ultratome III or Porter-Blum MT-2. Sections were stained with uranyl acetate and lead citrate and examined in an AEI EM6, AEI EM801, or Zeiss EM 9 electron microscope.

The kidneys of at least 4 animals were examined at each time period, and the ultrastructural description that follows is based on the study of 62 rats. The renal effects of a

¹The abbreviations used are: DMN, dimethylNitrosamine; RER, granular endoplasmic reticulum.

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protein-free diet were checked by preparation of untreated rats 1 and 2 weeks after commencement of the experimental diet and thereafter upon return to a conventional diet at the same weekly or monthly intervals as for treated rats. Altogether, 24 control rats were examined ultrastructurally.

RESULTS

The series of ultrastructural changes described below were recorded in all protein-depleted rats dosed with 60 mg/kg DMN. Similarly, all protein-depleted rats receiving 50 mg/kg DMN displayed the same range of acute renal changes, and chronic renal lesions were recorded in all but approximately 10%. In most rats fed a protein supplement and treated with 30 mg/kg DMN, mild alterations were seen in the acute phase, but chronic lesions persisted in only about 30% of the rats. The ultrastructural nature ofpersisting lesions in the latter group was identical with those in rats receiving the higher doses. Electron microscopic examination of untreated, control rats fed a protein-free diet for 2 weeks demonstrated normal appearance of renal tubules, glomeruli, blood vessels, and interstitial cells at all stages.

A detailed series of micrographs illustrating the light microscopic aspect of the development of DMN-induced renal interstitial lesions has been presented previously (8). For the sake of economy, electron micrographs in this paper, with 1 exception, will not be accompanied by an appropriate light microscope illustration. Where it may be particularly desirable to identify ultrastructural appearance with light microscope histology, reference will be made in the legend to a companion illustration in the previous paper.

Acute alterations induced by DMN in the convoluted tubules, although referred to, will be described in detail elsewhere (G. C. Hard and W. H. Butler, in preparation). The cortical intertubular space of the normal rat kidney is occupied by the endothelial cells of capillaries, cortical interstitial cells, and a few macrophages. Renal cortex interstitial cells (3, 18) are located in the triangular spaces between tubules and capillaries. They are narrow, elongate cells with long, attenuated processes, resembling the inactive fibrocyte (17, 22). The elongate nucleus has a clumped chromatin pattern with prominent peripheral condensations and often a small nucleolus. RER occurs in moderate amounts as parallel arrays of elongate cisternae, and the Golgi apparatus is relatively small. Below the plasma membrane, narrow tufts of microfilaments are present, particularly at the periphery of attenuated processes. In the vicinity of arterioles, some interstitial cells are plumper, with abundant, anastomosing profiles of RER and other organelles. Such cells resemble active fibroblasts (17, 22). Schwann cells and unmyelinated nerve fibers are also found in this location in the normal rat and, possibly, occasional mast and plasma cells (1). The interstitial cortex cell will be referred to as the cortical fibrocyte or fibroblast, depending on the degree of cytoplasmic development.

With the light microscope, alterations were not seen in the rat kidney until aggregations of interstitial cells began to develop around the hilus of a few glomeruli at 3 and 4 days. However, ultrastructural lesions were identified 24 hr after DMN administration in cortical fibroblasts associated with some glomeruli. This was not a conspicuous or widespread change. At the 60 mg/kg dose, altered cells, although present in all rats examined at this stage, were only very sparsely distributed throughout the cortex. At the 30 mg/kg dose, they were seen infrequently in some rats but not at all in others. The intracellular changes involved lipid droplet accumulation, formation of myelin figures, and sometimes nuclear disorganization and dilation of RER and nuclear envelope (Fig. 1). By 4 days, autophagic vacuoles were prominent in some cells (Fig. 2). In addition, occasional afferent arterioles showed alteration in most rats at the higher dose levels. Some granular epithelioid cells and smooth muscle fibers exhibited a loss of microfilaments, resulting in lowered cytoplasmic density, a mild dilation of the nuclear envelope, and frequent clear vacuoles, which represented dilated fragments of RER and mitochondria (Fig. 3). Very occasionally, a disintegrated myoid cell was observed in the arteriole wall as an aggregation of membranes up to 4 days after treatment.

In an attempt to confirm what appeared to be a specific action of DMN on the renal cortex interstitial cells, 6 additional protein-depleted rats were given injections of the lethal dose of 100 mg/kg and examined at 1 and 3 days. By 24 hr, most cortical fibroblasts in the vicinity of the majority of glomeruli displayed a range of intracellular alterations, including necrosis (Fig. 5). Cortical fibrocytes further removed from glomeruli also were affected frequently. Intracellular alterations were more severe than at lower dose levels; lipid droplets were more frequent, and autophagic vacuoles were larger. Segregation of the nucleolar components was a more prominent alteration (Fig. 6). By 3 days, this lethal dose of DMN was responsible for an almost complete necrosis of these cells.

The occasional aggregations of cells that began to form around glomeruli by 3 or 4 days were associated with dividing cells within the interstitial space and with a mononuclear cell infiltration. By 7 days, the interstitial reaction had reached a peak, and, as well as numerous discrete aggregations of cells associated with glomeruli, there was a diffuse increase in cells throughout the corticomedullary cortex. Large interstitial cells in mitosis, located in the close vicinity of the glomerular hilus, were seen as early as 2 to 4 days. Such cells were characterized by quite frequent, peripherally disposed profiles of RER and sparse tufts of microfilaments below the plasma membrane (Fig. 4). Some were associated with strands of collagen. These features indicated that these dividing cells were cortical fibrocytes or fibroblasts. Many mitoses seen at the peak of reaction proved on ultrastructural examination to be capillary endothelial cells (Fig. 7), but occasional dividing lymphoid cells were also noted. A number of capillary endothelial cells within prominent hypercellular foci were increased in size with a concomitant increase in cytoplasmic organelles, particularly polyribosomes (Fig. 8). Their nuclei were often of irregular shape. In some cases, extension of the endothelial lining beyond the vessel wall into the interstitial space was observed, suggesting formation of new capillary loops. Very rarely, endothelial cells were degenerate and contained autophagic vacuoles and lipid. At the height of interstitial response to
DMN, the predominant interstitial cell was the macrophage (Fig. 9). Monocyte infiltration through breaks in capillary walls was often observed. The macrophage was distinguished from the cortical fibrocyte by its fenestrated cell border resulting from the interconnection of numerous rounded cytoplasmic processes. Macrophages frequently contained lipid droplets and prominent phagolysosomes in which erythrocyte fragments and interstitial cell debris could be identified. The phagocytes were associated in particular with damaged tubule cells in which lipid droplets and autophagic vacuoles were also prominent. The intervening tubular basement membrane was thickened and contained clumps of clear and dense vesicles, membrane whorls, and basal cytoplasmic extensions from the adjacent damaged epithelial cells. Cortical fibrocytes were also conspicuous components of cell aggregations and were often associated with scant bundles of collagen. The extremely close morphological resemblance of some of these cells to endothelial cell extensions from capillaries made it impossible to distinguish between the 2 cell types on many occasions. Other cells possessed the abundant RER of fibroblasts. At 7 days, some of these cells still contained autophagic vacuoles and lipid droplets, while a small percentage with hydrated cytoplasm and markedly dilated RER appeared to be undergoing degeneration. Lymphocytes were also frequent in hypercellular foci.

In rats receiving 50 or 60 mg/kg DMN, the acute peak of interstitial reaction frequently persisted until 2 weeks. In rats treated with 30 mg/kg and fed a protein supplement, the acute reaction had disappeared by 2 weeks, and, in the few remaining hypercellular foci, lymphoid cells were the most frequent constituent. This was in contrast to protein-depleted rats treated with higher doses, in which the macrophage, as at 7 days, remained the dominant cell type at 2 weeks.

By 3 and 4 weeks, regression of lesions was well under way. Ultrastructural examination of persisting periglomerular foci showed that lymphocytes were predominant, and these frequently formed large clumps with still-numerous macrophages. The latter often contained ingested erythrocyte fragments and occasional degenerate cells. Cortical fibrocytes and a few plasma cells were also present. This stage was marked by the appearance of an occasional cell within hypercellular foci, which, although retaining some features of the normal cortical fibrocyte, differed in several respects (Figs. 10 to 13). Such unidentified cells were much larger, with more abundant cytoplasm and plumper processes extending for long distances through the lesions. The clear nucleus was large, lacking a clumped chromatin pattern and with a decreased peripheral density. The nucleolus was very prominent and hypertrophied. The cytoplasm contained many anastomosing profiles of RER, which frequently were dilated. In some, mitochondria were abnormal: irregular in shape, increased in density, or of giant size (Fig. 12). Narrow but conspicuous bands of microfilaments were present peripherally (Fig. 11, inset), and the plasma membrane was indented by occasional caveolae. Not infrequently, cells of this type were seen at the center of a compact clump of lymphocytes and plasma cells.

At 6 and 8 weeks, lymphocytes remained the predominant cell type in persisting periglomerular aggregations, although by now plasma cells were almost as numerous (Fig. 14). Cortical fibrocytes and endothelial cells remained difficult to distinguish one from the other, and new capillary formation seemed prominent. Unidentified cells retaining the morphology described in earlier foci were more frequent but nevertheless constituted only about 6% of cell numbers. Occasionally, they appeared as several layers of very elongate cells encircling a renal tubule (Fig. 17). More frequent were attenuated cell processes with hydrated cytoplasm and very dilated RER, which also closely encircled sequestered tubules (Fig. 15). In addition, large cell processes filled with microfilaments and displaying dense peripheral zones were seen (Fig. 16). These fibers, conforming to smooth muscle, were not associated with existing arteries or arterioles but, on occasion, were found to be cytoplasmic extensions from the large unidentified cell type. A small number of necrotic cells were still found in persisting lesions. Some of these appeared to have been mast cells; others were degenerate, unidentified cells.

Tubules and glomerular capsules within the persisting hypercellular foci were invariably surrounded by a thickened, tortuous basement membrane, which frequently contained vesicles, granules, and membrane profiles (Fig. 17). At the light microscope level, this material appeared as a thick, hyaline band surrounding the tubule. Microvilli were sparse at the luminal surface of sequestered tubule cells, and infoldings at the base were decreased or lost, but tufts of microfilamentous material were prominent (Fig. 15). The cytoplasm of these tubules often contained conspicuous autophagic vacuoles, myelin figures, and lipid droplets. Profiles of RER were frequently disposed in parallel arrays.

At 12 weeks, persisting hypercellular foci were much smaller, consisting almost entirely of clumps of large, abnormal cells in association with erythrocytes (Fig. 18). Lymphocytes, macrophages, plasma cells, and normal cortical fibrocytes were present only in very small numbers. The predominant cells of these lesions were morphologically very similar to the occasional, unidentified cells described in earlier foci, for the somewhat hydrated cytoplasm contained numerous profiles of RER filled with floccular material, and the clear nucleus lacked chromatin condensation (Fig. 19). Nucleoli were very prominent, and their structure was abnormal, including hypertrophy and sometimes fragmentation or disorganization of the components. Processes from adjacent abnormal interstitial cells were joined by intercellular junctions, thus enclosing spaces, which were invariably occupied by erythrocytes.

By 16 weeks, the microscopic neoplasms were larger. Tumor cells were of spindle and stellate shape and were morphologically identical with cells both in 12-week foci and in well-developed mesenchymal tumors seen at 20 weeks (Fig. 20). Spindle cells in the center were quite hydrated, while those at the lesion periphery were denser. Occasional smooth muscle fibers were seen in lesions at 12 and 16 weeks, and these were similar to those seen at 6 and 8 weeks. Capillary-like vessels were numerous, and erythrocytes were frequent in the interstitial space. Immediately beyond the lesion, attenuated tumor-cell processes infiltrated between tubules.

By 20 weeks, the cell aggregations were well developed.
displaying the variety of histological types characteristic of mesenchymal tumors, which included aggregations of spindle cells forming fibrosarcomatous sheets, stellate cells mimicking primitive mesenchyme, smooth muscle of vascular type, and the clumping of tumor cells to form vessel-like structures with cleft-like lumens. Sequestered tubules displayed the same range of alterations as those seen in tubules surrounded by the interstitial reaction shortly after DMN treatment. In addition, the lining cells of some tubules were undergoing hyperplasia. Other sequestered tubules and some Bowman’s capsules had become dilated to form very large cysts.

At no stage during the development of the interstitial lesions or in the tumors themselves were ultrastructural particles seen that could be identified as viral forms.

DISCUSSION

Ultrastructural examination of the series of changes induced by carcinogenic doses of DMN in the renal cortex interstitium of the rat proved to be of value, extending the observations already recorded with the light microscope. Within 24 hr of treatment, a few sparsely distributed fibroblast- and fibrocyte-like cells in the vicinity of glomeruli show intracellular changes; in particular, lipid accumulation, formation of autophagic vacuoles, and segregation of nucleolar components. The cytoplasmic changes are manifestations of intracellular degeneration (10). If the dose of DMN is increased to a lethal level, the specificity of action is enhanced, and cortical interstitial cells undergo widespread necrosis. Coincident with the interstitial damage, tubules, especially those in the vicinity of glomeruli, also show degenerative changes. Within a few days, interstitial cells ultrastructurally suggestive of cortical fibrocytes in the vicinity of the glomerular hilus are stimulated into division. Thus hypercellular foci that begin to develop at 3 to 4 days, reaching a peak at 7 to 14 days, result from the infiltration of mononuclear phagocytes and lymphocytes, together with a proliferation of cortical fibrocytes and capillary endothelium. The intensity of cell infiltration is related mainly to a macrophage response to tubule epithelial damage, although some large foci are also associated with damaged afferent arterioles and degenerate interstitial cells. Once the acute tubule changes have resolved, there is a rapid disappearance of the interstitial reaction, leaving relatively few periglomerular cell aggregations from 3 weeks onwards. At this stage, the lymphocyte is the dominant cell form, although plasma cells increase to become almost as numerous by 8 weeks. Appearing in very small but increasing numbers are very large cells with many of the characteristics of fibroblasts but which, because of their size, sometimes unusual mitochondria, clear nuclei with hypertrophied nuclei, and extensive hydrated processes often encircling tubules, constitute abnormal components of the renal cortex interstitial space. The presence of smooth muscle fibers free in intertubular areas is also abnormal. The tendency for a small percentage of cells to become necrotic within the lesions seems to be related to these abnormal cells. Their close association on occasion with clumps of lymphocytes and plasma cells suggests that an interaction between these cell types may be occurring. By 12 weeks, there is a sudden reduction in immunological cells in persisting foci, which now assume the form of aggregations of cells morphologically similar to abnormal cells of earlier lesions. Beyond this point, microscopic tumors at 16 weeks develop into macroscopic neoplasms, which display the characteristic spectrum of vascular differentiation (9).

Despite the high incidence of renal tumors following treatment with a single injection of DMN, acute structural damage in renal cells has not been recorded (13). Electron microscopy reveals that acute cellular damage is produced by a carcinogenic dose. It is significant that both epithelial cells and interstitial cells show ultrastructural signs of toxic injury, for DMN is able to produce independently both epithelial and mesenchymal tumors. Several points of correlation can be seen between the type of interstitial cell injured in the acute stages and ultimate development of mesenchymal tumors. The degree of cell injury observed with the electron microscope is dose dependent, and this correlates with the dose dependency of tumor incidence (7). The location of most altered cells next to glomeruli is highly significant, for accompanying light microscope studies have shown that mesenchymal tumors arise from persisting interstitial lesions associated with the glomerular hilus (8). No less important is the morphological similarity between the acutely injured cells and some of the differentiated cells of well-developed tumors. Many tumor cells have the appearance of fibroblasts with abundant, dilated RER and peripheral tufts of microfilaments (9). These, too, are the characteristics of affected periglomerular cells 24 hr after treatment, cells that appear to be activated forms of the more typical cortical fibrocyte. These observations support the proposal that in single-pulse carcinogenesis there is a relationship between acute intracellular damage and ultimate neoplastic transformation and development.

There can be no doubt that interstitial lesions persisting at 12 weeks in these studies are very small aggregations of tumor cells, for their morphology and organization is identical with that of microscopic tumors at 16 weeks. The nature of the large unidentified cells seen in earlier lesions is less certain, although their morphology and behavior indicate that they, along with the abnormal smooth muscle fibers, are early neoplastic forms. Their basic ultrastructure suggests derivation from fibroblast-like cells located near the glomerular arterioles. The ultrastructural sequence involved in the actual cellular transformation, however, is under further study. These abnormal cells are not only few in number but are obscured in the early stages by the intense inflammatory response. The rapid ascendancy of tumor cells in persisting lesions at 12 weeks with a concurrent disappearance of immunological cells suggests that expression of immunological surveillance (2) is abruptly impeded.

Electron microscopic examination has demonstrated the vascular nature of renal mesenchymal tumors (9). Pericyte-like cells, vascular smooth muscle, and the tendency for tumor cells to form vessel-like channels are some of their prominent features. These observations are confirmed by the nature of microscopic tumor-cell aggregations at 12 and 16 weeks. The propensity for adjacent cells united by intercellular junctions to enclose interstices occupied by erythrocytes emphasizes
their vasoformative behavior. The presence of smooth muscle fibers similar to the vascular smooth muscle forms of the developed tumor is further evidence of the early ability to differentiate into vascular components. These vasoformative properties of tumor cells may not be incompatible with the identity of the interstitial cell injured in the acute phase of DMN toxicity. During renal embryogenesis, a few mesenchymal cells constantly accompany the capillary that grows into the cleft of each metanephric vesicle (19, 26). Such capillaries are direct outgrowths of adjacent vessels, which richly vascularize the metanephric blastema. The accompanying mesenchymal cells appear to have the properties of pericytes (20), for they differentiate into mesangial cells of the glomerulus and smooth muscle cells of the glomerular arterioles. It is not unlikely, then, that at least some interstitial cells remaining in the adult renal cortex might retain the ability to function as vascular precursors.

This ultrastructural study demonstrates that DMN-induced renal mesenchymal tumors originate from interstitial cells with fibroblast morphology in the vicinity of the glomerular hilus, cells that within 24 hr of carcinogen treatment may demonstrate signs of intracellular injury. Abnormal cells can be recognized within interstitial aggregations as early as 3 weeks, and by 6 weeks some of the differentiated cell forms characteristic of the tumor may be present. Transformation of malignant cells into epithelial components was not observed in microscopic neoplasms. Epithelial structures in chronic lesions characteristic of the tumor may be present. Transformation of malignant cells into epithelial components was not observed in microscopic neoplasms. Epithelial structures in chronic lesions associated with tumor cell aggregations are stimulated to hyperplastic proliferation, accompanying mesenchymal cells appear to have the properties of pericytes (20), for they differentiate into mesangial cells of the glomerulus and smooth muscle cells of the glomerular arterioles. It is not unlikely, then, that at least some interstitial cells remaining in the adult renal cortex might retain the ability to function as vascular precursors.

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mitochondrion (M3) is of normal appearance. The cytoplasm of this cell contains many anastomosing channels of RER, and the cluster pattern of to be of giant size. Both mitochondria were visible as granules within the cytoplasm of this cell with relatively low-power light microscopy. The 3rd prominent, sometimes dilated RER (arrows). Adjacent cells are united by intercellular junctions and enclose small spaces that are occupied by erythrocytes (RBC). The macrophage (Mp) contains phagocytized fragments of red blood cells. Ly, lymphocyte; Lu, capillary lumen; DT, distal collapsed, and the lumen is obliterated. Autophagic vacuoles (AV) are numerous, and some are very large. The tubule basement membrane (BM) is discontinuous. A large autophagic vacuole containing myelin figures (AV) and lipid droplets (Li) can still be identified. Art, afferent arteriole; Tb, tubule. x 11,000.

Fig. 6. Cortical fibrocyte (CF) 24 hr after administration of 100 mg/kg DMN. The nucleolus shows marked segregation of its components (arrow). A lipid droplet (Li) is seen in the cytoplasm. Lu, capillary lumen. x 18,600.

Fig. 7. Capillary endothelial cell (En) in mitosis 7 days after administration of 50 mg/kg DMN. Arrowheads, basement membrane; Lu, capillary lumen. x 12,800.

Fig. 8. Capillary endothelial cell (En) within hypercellular focus 7 days after administration of 60 mg/kg DMN. The cell is increased in size, and there is a hypertrophy of organelles, particularly Golgi (G) and polyribosomes. Lu, capillary lumen; Tb, tubule. x 18,500.

Fig. 9. Survey of a periglomerular, hypercellular focus 6 days after administration of 60 mg/kg DMN. The interstitial cells are mainly monocytes or macrophages (Mp) and lymphocytes (Ly), although a few cytoplasmic processes of cortical fibrocytes (arrows) are also seen in this field. The proximal tubule (PT) shows lipid accumulation (Li) and a reduction of brush border and basal infoldings. x 4,000. (See Ref. 8, Fig. 3, for corresponding light micrograph.)

Fig. 10. Survey of a periglomerular cell aggregation 3 weeks after administration of 50 mg/kg DMN. Ly, lymphocytes; Mp, macrophages; arrows, cortical fibrocyte processes. Two abnormal cells (AC) with large and, in 1 case, irregular nuclei are present. The nuclei of these cells are enlarged, and the nuclei lack a clumped chromatin pattern. Their cytoplasm contains numerous profiles of RER. Small tufts of collagen (C) are sparsely scattered through the lesion. Lu, capillary lumen; Tb, tubule; BM, basement membrane. x 4,000. (See Ref. 8, Fig. 6, for corresponding light micrograph.)

Fig. 11. Large abnormal cell (AC) in hypercellular focus persisting 3 weeks after administration of 30 mg/kg DMN. Either the cell is binucleate or the nucleus is very elongate and sectioned twice. The nuclei are enlarged. The amplt cytoplasm is filled with organelles, including RER and vesicles. There is intimate contact between the plasma membrane of this cell and 3 macrophages (Mp). In several areas, narrow bands of microfilaments (arrows) are present. In the plasma membrane. The details of one of these is shown in the inset. At this higher magnification (x 41,500), dense zones (arrowheads) are present in the microfilamentous band. Tb, tubule. x 7,000. (See Ref. 8, Fig. 10, for corresponding light micrograph.)

Fig. 12. Abnormal cell in hypercellular focus 4 weeks after administration of 50 mg/kg DMN. Two abnormal mitochondria are present (M1, M2). These organelles are greatly increased in density, and 1 (M1) is very large and of abnormal shape. The 2nd (M2) proved on serial section section also to be of giant size. Both mitochondria were visible as granules within the cytoplasm of this cell with relatively low-power light microscopy. The 3rd mitochondrion (M3) is of normal appearance. The cytoplasm of this cell contains many anastomosing channels of RER, and the cluster pattern of numerous ribosomes (arrowheads) associated with these profiles is characteristic of fibroblasts. Beneath the plasma membrane, tufts of microfilaments (arrows), C, collagen. x 26,000.

Fig. 13. A very large abnormal cell (AC) within a hypercellular focus 3 weeks after administration of 50 mg/kg DMN. The cytoplasm of this cell is abundant and projects into an extensive process, which ramifies some distance across the lesion between a proximal tubule (PT) and an arteriole (Art). The cytoplasm is filled with RER, and some lipid droplets (Li) are also present. Arrowhead, caveola that indents the plasma membrane. Mp, macrophage; CF, cortical fibrocyte. x 7,500.

Fig. 14. Survey of a hypercellular focus 8 weeks after administration of 60 mg/kg DMN. Lesions at this stage consist predominantly of lymphocytes (Ly) and plasma cells (PC). Macrophages (Mp) and processes of cortical fibrocytes (arrows) are also present. x 4,300.

Fig. 15. Interstitial lesion persisting 8 weeks after administration of 30 mg/kg DMN. An abnormal cell process (AC) with hydrosapstomatized and dilated profiles of RER (ER) closely encircles a sequestered tubule. Collagen (C) is associated with this cell. Note the difference in size between the abnormal cell process and the processes of normal cortical fibrocytes (CF). Condensations of microfilaments (MF) are visible at the base of the tubule epithelium. BM, tubule basement membrane; En, capillary endothelium. x 14,000.

Fig. 16. Intestinal lesion 8 weeks after administration of 60 mg/kg DMN. Several abnormal cell processes (AC) are present. These are filled with microfilaments, which, together with the peripheral dense zones (arrow), identify them as smooth muscle. En, capillary endothelium; Lu, capillary lumen. x 17,500.

Fig. 17. Intestinal lesion persisting 8 weeks after administration of 60 mg/kg DMN. A tubule (Tb) sequestered within the hypercellular focus is collapsed, and the lumen is obliterated. Autophagic vacuoles (AV) are numerous, and some are very large. The tubule basement membrane (BM) is very tortuous and thickened. Two abnormal cells (AC) with clear nuclei closely encircle the collapsed tubule in a fashion characteristic of cells in advanced tumors. Ly, lymphocytes; CF, cortical fibrocyte. x 4,000. (See Ref. 8, Fig. 9, for corresponding light micrograph.)

Fig. 18. Phase-contrast light micrograph of a small, persisting lesion 12 weeks after administration of 50 mg/kg DMN. The lesion consists mainly of abnormal cells (AC) with clear nuclei and prominent nucleoli, interspersed with free erythrocytes (RBC). PT, proximal tubule; Mp, macrophage. x 1,350.

Fig. 19. Electron micrograph of the same lesion depicted in Fig. 18. The abnormal cells (AC) have clear nuclei, hypertrophied nucleoli, and prominent, sometimes dilated RER (arrow). Adjacent cells are united by intercellular junctions and enclose small spaces that are occupied by erythrocytes (RBC). The macrophage (Mp) contains phagocytized fragments of red blood cells. Ly, lymphocyte; Lu, capillary lumen; DT, distal tubule; PT, proximal tubule. x 4,000.

Fig. 20. A portion of a microscopic tumor 16 weeks after administration of 50 mg/kg DMN. Three spindle-shaped tumor cells (T1, T2, T3) are present. In 1 (T1), there is a very prominent, hypertrophied nucleolus (arrow). The 2nd (T2) contains numerous profiles of RER (ER) filled with dense, floccular material. Polyribosomes are also numerous. Sequestered between the tumor cells is a preexisting, compressed tubule (Tb). Basement membrane (arrowheads) surrounding this tubule is intact. x 12,900. (See Ref. 8, Fig. 12, for corresponding light micrograph.)
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