AUTORADIOGRAPHIC ANALYSIS OF PROLIFERATIVE ACTIVITY IN RAT KIDNEY EPITHELIAL AND MESCENHYMAL CELL SUBPOPULATIONS FOLLOWING A CARCINOGENIC DOSE OF DIMETHYLNITROSAMINE

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SUMMARY

In a system that yields 100% incidence of renal mesenchymal tumors and a 30 to 40% incidence of renal cortical epithelial neoplasms, the proliferative activity of renal epithelial and mesenchymal cell subpopulations following a single dose of dimethylnitrosamine (DMN) was traced by autoradiographic analysis of [methyl-3H]thymidine uptake during the 3 weeks immediately posttreatment. The initial response to DMN was a depression in DNA synthesis and mitosis to near 0 levels in all segments of the nephron and in attendant mesenchymal cells for a period of 1 to 3 days. Following the period of inhibition, increased DNA synthetic activity was observed in certain subpopulations of both epithelium and mesenchyme and these patterns were matched by equivalent mitotic activity. A stimulation of DNA synthesis was observed in cells of the proximal and distal tubules of Zones 1 and 2 but in no other epithelial segments. The increased activity was most intense in Zone 1 epithelium reaching a peak at the 10th day after DMN injection, 4 days after epithelial cell necrosis had commenced.

In renal mesenchyme, the major response involved only the interstitial cells of Zones 1 and 2. At Day 3, there was a wave of increased DNA-synthetic and mitotic activity in the free interstitial cells of the cortex, followed by a 2nd, more intense peak of activity at Day 6. The cells responding at Day 3 appeared to involve the resident population of cortical fibroblasts while the major contribution to the Day 6 peak came from infiltrating mononuclear inflammatory cells, although resident fibroblasts and capillary endothelium also contributed. A significant wave of increased activity involved the interstitial cells of Zone 2, but the peak, although of equivalent intensity to the response in Zone 1, was single and occurred 3 days later at Day 9. Apart from a small, brief, and variable wave of activity in interstitial cells of Zone 3 from Days 8 to 10, no other mesenchymal cell populations in the kidney were stimulated by the injection of DMN.

The carcinogen therefore exerted its most significant effect on those epithelial and mesenchymal cell subpopula-

INTRODUCTION

When a single i.p. injection of DMN, 60 mg/kg, is administered to rats that have been preconditioned by having been fed a diet high in carbohydrate but lacking in protein, every survivor of the acute toxicity develops renal neoplasia (8, 19, 26). Both mesenchymal and epithelial tumors are induced, the former in very high incidence approaching 100% and the latter at a lower incidence of 30 to 40% (8). A system combining a single dose of carcinogen with a high induction of cancer has obvious advantages for the study of the cancer process and the DMN-induced renal mesenchymal tumor in particular is a most suitable experimental cancer model.

Sequential studies using light and electron microscopy have indicated that the mesenchymal tumors of the rat kidney can be identified first as small aggregations of mesenchymal cells in the intertubular spaces in the cortex, or more frequently at the corticomedullary junction at 12 to 16 weeks after treatment (9, 10). The studies have focused attention, therefore, on cell types resident in the interstitium as likely candidates for cells of origin of this particular tumor.

On the other hand, the early epithelial tumors consist of tubule cell proliferations, usually identifiable by 12 weeks and restricted to the cortex, particularly in close proximity to glomeruli (13). Ultrastructurally, some of these neoplasms present various features of proximal tubule epithelium including aberrant brush border and peroxisomes (12), while sequential studies of their development confirm that a proportion of the early lesions take origin within the 1st segment of proximal convoluted tubules (13). However, these observations did not exclude the possibility that epithelial tumors may arise also from distal tubule epithelium.

DMN is undetectable after 19 hr in rats preconditioned by a protein-free diet (26) so that any stimulation of cell proliferation occurring as an early response within the 1st weeks of DMN administration is likely to be related to the

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carcinogenic process. A study of cell kinetics during the acute reaction of the kidney to the carcinogen in a system where a single pulse leads to a high incidence of neoplasia may help to identify the specific target cell type from which the tumor cell nests ultimately will be derived.

In the 1st 2 to 3 weeks following the administration of a single carcinogenic dose of DMN, a diffuse inflammatory response occurs in the cortex of the kidney which appears to be associated with damage to the convoluted tubules of that zone. The injury is most marked at the end of the 1st week but the inflammation usually subsides by the end of the 3rd week. There follows a time lapse of some weeks before the early foci of neoplastic cells can be recognized.

By means of autoradiography, this acute phase of the response of the kidney to DMN has been examined further in an attempt to quantitate the extent to which the single carcinogenic dose influences kidney cell proliferative activity. In a previous report it was shown that DMN does elicit an increase in DNA synthesis involving both mesenchyme and epithelium of the outer zones of the rat kidney (14). The information has been augmented by the examination of additional animals and by analysis of the data in terms of cell form or type and spatial distribution within the parenchyma and interstitium. In this communication, the proliferative activity of epithelial and mesenchymal cell subpopulations has been assessed to determine which segments of the nephron and attendant cells are involved and whether the sites of this early reactivity can be related to ensuing tumor development.

**MATERIALS AND METHODS**

**Animals.** Random-bred male and female rats of Porton albino Wistar stock derived from a breeding nucleus imported from the Laboratory Animals Centre, Carshalton, U. K., were housed in plastic cages and maintained on a conventional pellet diet with water ad libitum. For 3 days prior to carcinogen administration, 5- to 6-week-old rats were placed in wire grid cages and fed a powdered diet consisting exclusively of a 50:50 glucose:sucrose mixture with 20% aqueous glucose solution as drinking water.

**Chemicals.** DMN (Eastman Organic Chemicals, Rochester, N. Y.) was purified by distillation and administered as a 3.0% solution in 0.9% NaCl solution. [3H]TdR in sterile water (specific activity, 20 Ci/m mole) was obtained from New England Nuclear, Boston, Mass.

**Experimental Plan.** After 3 days of being fed the glucose:sucrose powder, the rats were given a single i. p. injection of DMN in 0.9% NaCl solution at a dose of 60 mg/kg body weight and returned to conventional housing and diet. The rats used as controls were given a 0.2-ml i. p. injection of 0.9% NaCl solution following the carbohydrate dietary schedule. At 24-hr intervals after DMN or 0.9% NaCl solution-only treatment, the rats were given a single i. v. pulse of [3H]TdR via the saphenous vein at a dose of 1.0 μCi/g body weight. This injection was made between 10 and 10:30 a.m.

One hr after the pulse of [3H]TdR, the animals were anesthetized with ether and prepared for in vivo renal perfusion with fixative. Following perfusion for 10 to 15 min with 1% glutaraldehyde in 0.14 M Veronal acetate buffer containing 2% w/v dextran T40 (Pharmacia, Uppsala, Sweden) the hardened kidneys were removed and sectioned in the sagittal plane into 1 mm slices. Postfixation in 1% buffered (0.14 M Veronal acetate) osmium tetroxide for 16 hr was followed by dehydration through graded alcohols and embedding in Spurr’s resin (24).

**Autoradiography.** Large-area sections of kidney were cut at 1 to 2 μm with an LKB-modified Cambridge Huxley ultramicrotome. Such a thickness incurred a labeling error of less than 10% in relation to the disparity in nuclear dimensions of differing cell types and the 3H autoradiographic range (20). Sections on glass slides were dipped mechanically in Ilford L4 nuclear track emulsion, exposed for 4 weeks at room temperature, and developed in Kodak Microdol-X developer.

For photographic illustration of the autoradiographs a method of double exposure was used. Selected fields were photographed through a 40× objective first using a 0.8:0.95 dry-field condenser (Carl Zeiss Inc., New York, N. Y.), with the granules of nuclear label in focus and then under phase-contrast optics with the focus set in the plane of the tissue. The resultant exposure demonstrated the grains of nuclear label in positive prints as aggregations of white dots without loss of cellular detail.

**Quantitation of Data.** The autoradiographically prepared sections were examined without staining under phase-contrast optics. Labeled cells, mitotic figures, and total cell numbers were scored in high-power fields at ×500. Rather than interpret the data in the arbitrary unit of microscopic fields, an attempt has been made to relate the information at least partially to actual kidney dimensions by assessing percentages of labeled nuclei of various cell populations in a unit kidney section. Estimates of cell numbers constituting subpopulations of various zones of a representative unit section were determined by counting cells in high-power fields and relating the counts in this constant area to the total areas occupied by each zone in a unit slice. Mean zonal areas per unit slice were assessed by planimetric measurement of accurate photographic reproductions of sagittal sections cut through the median plane of each of 3 kidneys. The variation in cell counts per high-power field of different subpopulations between control rats and test rats with uninfamed kidneys was usually of the order of 20% or less.

In assessing proliferative activity, at least 3 DMN-treated rats were examined at each time interval, that is at Days 1 to 10, 14, and 21. The control group consisted of 5 0.9% NaCl-treated rats prepared at intervals ranging from 1 to 14 days after termination of the carbohydrate diet. Proliferative activity in each rat was assessed by scoring labeled cells and mitotic figures in an average of 100 fields in Zone 1, 40 in Zone 2, 40 in Zone 3, and 25 in Zones 4 and 5 combined. Thus, each point on the illustrative charts represents the mean determination from no less than 3 rats qualified by the standard deviation of the group. Each chart depicts the control range as ± 1 S.D. derived from the group data of 10 rats. Student’s t test was used for assessing the statistical significance of the variation between test and control groups of rats.
RESULTS

The rat kidney is a unilobar organ that, according to the distribution of segments of the nephrons, can be divided into 5 zones (18, 27). Zone 1 (the cortex) consists of the convolutions of the proximal tubules (Segments 1 and 2), the distal convoluted tubules, some collecting tubules, and glomeruli. Zone 2 consists of the pars recta of the proximal tubules (Segment 3), the straight portions of the distal tubules, and collecting tubules. Zones 3 and 4 contain the thin descending limbs of Henle, the thick ascending limbs, and collecting tubules; while Zone 5, the papilla, consists of branched collecting ducts and the longest of Henle’s loops. The delineation of these various zones is illustrated in a perfusion-fixed section of whole kidney in Fig. 1.

Enumeration of Epithelial Cells

Glomerular Epithelium. The labeling indices of epithelial cells of the glomeruli, i.e., of Bowman’s capsule cells and epithelial cells of the tuft, were very low in control rats. Apart from an initial depression to 0 levels for the 1st 2 days, the number of epithelial cells labeled in glomeruli did not vary from the normal range over the 3-week period of study.

Proximal Tubule Epithelium. Chart 1A depicts the graphed data obtained by scoring labeled proximal tubule cells in Zone 1, and Chart 1B shows those in Zone 2. DMN induced a depression of DNA-synthetic activity to near 0 levels for the 1st 2 days in cortical proximal tubule cells followed by a return to normal levels for Days 3 and 4. At Day 5 there was an increase in activity which reached a peak at Day 10. At this point, labeled cells of proximal convoluted tubules (Fig. 2) were 17.4 times more frequent than the mean control value ($p < 0.01$) representing 2% of the total subpopulation. The increased response involved cells of both 1st and 2nd segments. Although the number of labeled cells had fallen by Day 21, the activity was still significantly elevated at this stage.

In Zone 2 (Chart 1B) DMN caused a depression of DNA-synthetic activity on the 1st day in cells of the pars recta. There followed a return to normal activity for the next 7 days with an increase in DNA synthesis reaching a peak at 10 days and declining to the normal range by 21 days. Compared to the activity in Zone 1 the peak value was low, being only 4.6 times higher than the mean of the control values and involving approximately 1% of pars recta cells.

Distal Tubule Epithelium. The graphed data for distal tubules of Zones 1 and 2 appear in Chart 2. A and B, respectively. In general, the pattern of DNA synthesis in distal tubules followed a similar course to that involving the proximal tubules. In both zones, there was a depression of activity in distal tubule epithelium for the 1st 3 days with an increase in activity commencing around the 5th day. The peak of increased response once again occurred at Day 10 and the activity had returned to normal by Day 21. In Zone 1, the peak of activity was 11.8 times higher than the control mean value ($p < 0.01$) representing approximately 2% of the subpopulation but only 5.4 times higher in Zone 2 ($p < 0.01$) where less than 1% of cells were involved. Labeled distal tubule cells in Zone 1 are illustrated in Fig. 3.

Henle’s Loops and Collecting Tubules. Neither cells of Henle’s loops nor those of collecting tubules, found in Zones 3, 4, and 5, were stimulated to engage in increased DNA synthesis in response to DMN. Apart from a depression of activity to near 0 levels for the 1st 3 days following DMN administration, the number of labeled cells remained within the normal range. This is illustrated in Chart 3 where the data for these different segments of the nephron are presented collectively. No significantly consistent increased response was noted in collecting tubule epithelium in Zones 1 or 2.

Counts of Mitotic Figures. In all zones the epithelial mitotic counts matched the pattern demonstrated by $[^3H]Tdr$ incorporation, confirming that isotope labeling was indicative of proliferative activity. In Chart 4, where the mitotic index of epithelial cells of the cortex is depicted, comparable to the labeling indices of proximal and distal convoluted tubules (Charts 1A and 2A), there was a depression in activity for the 1st 2 days with an increase in mitotic figures commencing at Day 5. The increased mitotic activity reached a peak at Day 10 when it was 14.9 times higher than the mean control value ($p < 0.01$), and by Day 21 the activity had returned to near normal.

Enumeration of Interstitial Cells

Quantitation of the Inflammatory Reaction. For 3 days following DMN administration, cell numbers in all zones of the kidney remained within the normal range but at the 4th
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Chart 2. Effect of 60 mg/kg DMN on the uptake of [3H]Tdr by distal tubule epithelium. A, Zone 1 cells; B, Zone 2 cells.

Chart 3. Effect of 60 mg/kg DMN on [3H]Tdr uptake by the epithelial cells of Zones 3, 4, and 5, i.e., Henle's loops and collecting tubules.

Chart 4. Effect of 60 mg/kg DMN on mitotic activity in the proximal tubule and distal tubule epithelium of Zone 1.

Chart 5. Effect of 60 mg/kg DMN on cell numbers in the interstitial space of Zone 1 of the rat kidney. The data are expressed as a percentage of the normal cellular content.

exception of 1 individual rat in which Zone 2 was involved at Day 10 (involving an increase in free cells from a base count of 16,770 to 33,150 per unit section), no variation in the numbers of cells in the intertubular spaces was evident in any of the other kidney zones. Thus, the inflammatory response was restricted to the cortex.

Interstitial Cells of Zone 1. The sequence of autoradiographic labeling involving the free cells of the intertubular spaces of Zone 1 is depicted in Chart 6A. For the 1st 2 days there was a depression of DNA synthesis most marked at Day 1 followed by a sudden increase beyond the normal range in the numbers of labeled cells at Day 3 (Fig. 4). At this point 3% of the 27,300 free interstitial cells in a unit day the numbers of interstitial cells in Zone 1 commenced to increase rapidly to Day 6, reaching a peak at Day 10, and thereafter declining to near normal levels by Day 21 (Chart 5). At the peak of the response, the cortical intertubular space contained 4 times as many cells (of relatively diffuse distribution) as the interstitium of control rat kidneys. This represented an increase from the base count in Zone 1 of 27,300 free interstitial cells per unit section to 109,060. Morphologically, the cells contributing to the increase were macrophages and lymphocytes consistent with the electron microscopic findings in a previous study (10). With the
section were labeled and the predominant cell type involved at this stage was elongated with well-developed cytoplasm, consistent with the morphology of the resident cortical fibrocyte (Fig. 5). After Day 4 there was a rapid linear increase in the number of free cells of Zone 1 involved in DNA synthesis to Day 6 with a rapid falloff in activity after Day 7. The peak value at Day 6 was 13.7 times the mean control \((p < 0.01)\). By Day 14 the activity had returned to near normal values and was within the control range by Day 21. The peak of DNA-synthetic activity coincided with a marked increase in the number of free cells in the intertubular spaces of the cortex, so that 9% of the 79,940 (mean value) free interstitial cells per unit slice in Zone 1 were labeled at Day 6. Labeled cells were particularly frequent in the proximity of glomeruli (Fig. 6). Morphologically, many of the active cells at Days 6 and 7 appeared to have the form of mononuclear inflammatory cells, lymphocytes, and mononuclear phagocytes (Fig. 7) in contrast to the predominant cell type labeled at Day 3. Marked cuffing by infiltrating mononuclear inflammatory cells of arteries and veins at the deep boundary of the cortex was characteristic at Days 6 and 7, and many of these cells were also labeled (Fig. 7). In particular, the technique of perfusion-fixation demonstrated clearly the margination of mononuclear cells along vessel walls in these and other areas of the cortex preparatory to migration through the endothelial lining into the tissue spaces (Fig. 8). A few of the marginated cells, mostly monocytes, in vessel lumens also displayed nuclear label. In contrast, the preliminary flush of heparinized 0.9% NaCl solution during intravascular perfusion-fixation cleared all leukocytes from the vessel lumens in control and noninflamed kidneys (Fig. 9).

At Day 10, when the number of free interstitial cells in the cortex was at its peak, the DNA-synthetic activity was declining rapidly. This is illustrated in Fig. 10 where, despite the crowded inflammatory cells between tubules, only 1 shows nuclear label.

Chart 6B illustrates the count of mitotic figures in interstitial cells of Zone 1. The form of the graph is identical to that for DNA synthesis, with a marked depression of mitotic activity for the 1st 2 days, a lesser but marked peak at 3 days, a major peak at 6 days, and a reversion to normal activity by the 10th day.

**Interstitial Cells of Zone 2.** The activity of free cells in the interstitium of Zone 2 is depicted in Chart 6C. Again there was a depression of DNA-synthetic activity following DMN treatment for 2 days with a return to normal at the 3rd and 4th day. There followed an increase in activity (Fig. 11)
reaching a peak at Day 9, i.e., 3 days later than the equivalent cells in Zone 1. The number of labeled cells had returned to within normal limits by Day 21. At the Day 9 peak, labeled cells were 14.3 times more numerous than in control kidneys \( p < 0.001 \) representing 6% of the estimated 16,770 free interstitial cells present in Zone 2 of a unit section. Morphologically, the Zone 2 cells involved in increased nuclear activity appeared to be both resident fibrocytes and other mononuclear cells, although accurate discrimination between these cell types was difficult at the light microscope level. As in Zone 1, some margination of leukocytes in small vessels was observed (Fig. 11).

**Interstitial Cells of Zones 3, 4, and 5.** DNA synthesis in interstitial cells of the 3 inner medullary zones was depressed for the 1st 2 days after DMN dosing. In cells of Zone 3 (Chart 6d) there was a brief increase in activity from Day 8 to Day 10 but the peak of increase was only 4.0 times above the control mean value \( p < 0.01 \) and involved 1.5% of the estimated 28,050 Zone 3 interstitial cells in a unit section. In contrast to the reaction of cells in the 2 outer zones, this increased response was variable within groups, some individual rats at this stage showing activity within the normal range.

No significant increase in DNA-synthetic activity was observed in the interstitial cells of Zones 4 and 5 (Chart 6E) during the 3-week period of study.

**Mesangial and Related Cells.** The labeling response of the mesangium is depicted in Chart 7A. Apart from a depression of activity at Day 1, DNA synthesis in mesangial cells remained within the normal range.

Labeling of smooth muscle fibers of the afferent and efferent arterioles was rare and not observed in cells of the juxtaglomerular bodies.

**Endothelial Cells.** The labeling activity of endothelial cells in Zone 1 is shown in Chart 7B. Following a depression to near 0, the number of labeled cells was elevated from Day 4 and remained above the normal range at Day 21. The only endothelial cells engaged in this increased activity were capillary lining cells of the intertubular spaces, not those of glomerular tufts or arterioles. Discrimination between capillary endothelium and the free interstitial cells during the inflammatory period was difficult at the light microscope level resulting in a wide variation in observations between animals.

Endothelial cells of Zones 2, 3, 4, and 5 were also scored but showed no consistently significant variation from the normal range of activity.

**DISCUSSION**

The distribution of epithelial segments of the nephron constitutes the basis for division of the rat kidney into zones as described in “Results.” However, a comment concerning the distribution of various subpopulations of mesenchymal cells through the kidney is necessary. The predominant resident cell in Zones 1 and 2 occupies the triangular spaces between the tubules and the endothelial lining cells of capillaries (2, 22). Most of these free cells have the ultrastructural morphology of relatively inactive fibrocytes but some, particularly in the vicinity of glomerular arterioles, possess features of more active fibroblasts (10). In addition, a few sporadic mononuclear cells resembling macrophages are scattered through Zones 1 and 2. Associated with arterioles in Zone 1 are unmyelinated nerve fibers supported by Schwann cells. The mesenchymal cells of the juxtaglomerular bodies are modified vascular smooth muscle cells related to and continuous with the mesangial cells of the glomerular tufts (16). In the human at least, the mesangial cells and smooth muscle fibers of arterioles are derived from the same mesenchymal cell precursors that constantly accompany the capillaries that grow into and vascularize the metanephric vesicles (28). In Zones 3, 4, and 5 the predominant resident cell is a fibroblast-like cell which contains lipid droplets and is ultrastructurally distinct from the resident cell of Zones 1 and 2 (1, 22). Because arachidonic acid constitutes a significant fraction of the isolated lipid droplets, it has been suggested that the cells may function as a storage site for prostaglandin precursor (21). In Zones 3 and 4, Bohman (1) has identified 2nd cell type of rounded form resembling agranulocytes, distinct from the lipid granule-containing cells. Pericytes, supported by basement membrane are also found in Zones 3 and 4 associated with the vasa recta.

As far as was possible, each of the various epithelial and mesenchymal cell subpopulations was examined for nuclear labeling and, although difficulty occurred in discrimination between certain cell types at certain stages (as detailed below), perfusion fixation and plastic embedding facilitated this identification. The degree of cell discrimination achieved would not have been possible with conventional fixation and embedding techniques. During the period of renal inflammation it was not possible, with consistent
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accuracy, to distinguish between resident interstitial cells and infiltrating mononuclear inflammatory cells. Even in the noninflamed intertubular spaces of normal rats, discrimination between resident mesenchymal cells and the small numbers of macrophage-like cells would require resolution by electron microscopy. Hence the relevant data for these particular populations have been recorded in terms of free interstitial cells and not according to specific cell type. However, in control rat kidneys and in those of test rats preceding the influx of inflammatory cells, it can be confidently concluded on the basis of the electron microscopical studies cited (2, 10, 22) that the vast majority of the estimated 27,300 free interstitial cells in Zone 1 of a unit kidney section and of the 16,770 in Zone 2 would represent resident cortical fibrocytes.

Mitotic counts were exactly comparable to the pattern of nuclear labeling so that [3H]Tdr uptake can be taken as a measure of cell proliferation. The very initial response to DMN is a severe depression to near 0 levels in proliferative activity affecting all epithelial and mesenchymal cells of the kidney regardless of zonal distribution. Depending on cell population, inhibition lasts from 1 to 3 days, the epithelial cells distal to the proximal tubule being affected for the longest period. This initial blanket effect of DMN on renal mesenchyme and epithelium alike appears to be the in vivo correlate of an in vitro property believed to be common to carcinogens in general, that of inducing growth inhibition and depression of mitotic activity in cultured rat kidney cells (15).

Both epithelial cell and mesenchymal cell subpopulations are involved in the stimulation of DNA-synthetic activity that ensues and these patterns will be discussed separately. In the nephron, the increase in DNA-synthetic activity which commences at Day 5 involves only the cells of the proximal and distal tubules, this response having greater intensity in Zone 1 than in Zone 2. Cells of Henle’s loops and collecting tubules are not affected. Thus the major stimulatory effect of the carcinogen is restricted to those specific tubule segments that previous studies indicate to be the most likely origin of the epithelial tumors. These are destined to develop weeks later in 30 to 40% of surviving others may arise from distal tubule epithelium (8, 12, 13).

The marked lag between administering a single pulse of carcinogen, full expression of epithelial cell injury, and peak of resultant proliferative activity makes the response of the rat kidney to DMN unique. The 1st structural signs of toxicity occur in cells of the proximal tubules within 24 hr of DMN injection (13), and this is seen as an accumulation of lipid droplets affecting particularly the 1st segment of a majority of nephrons. By 3 to 4 days, aggregations of apparent cytoplasmic debris develop beneath injured proximal tubule cells, associated with basement membrane convolution and thickening. Sporadic cell necrosis in cortical tubules follows and is most obvious around Days 6 and 7. The present study shows that the peak of proliferative activity, however, is delayed until Day 10 after carcinogen administration. This prolonged sequence of injury is difficult to explain in view of the fact that in this system DMN is no longer detectable in body fluids 19 hr after injection (26). However, it is accepted that DMN is not the proximate carcinogen but requires metabolic conversion within susceptible tissues to a biologically active intermediate (17).

The prolonged response nevertheless contrasts with the reaction of kidney epithelium to other nephrotoxins and to mechanical injury as assessed by comparable autoradiographic methods. Thus, a single injection of the renal carcinogen lead acetate stimulates a sharp peak of increased DNA synthesis in tubule epithelium at 30 hr in the rat and at 33 hr in the mouse, followed by a rapid decline to near normal by Day 3 (5, 7). Similarly, folic acid induces epithelial cell degeneration within 24 hr (4) followed by a peak of increased DNA synthesis at 36 hr and a sharp fall to near normal by Days 3 to 4 (3). The effects of mechanical injury, unilateral nephrectomy (5), and stripping of the renal capsule (6) reproduce the same pattern of proliferative activity.

Turning to the consideration of mesenchymal cell subpopulations, this autoradiographic study has confirmed the initial qualitative findings (9, 10) that DMN elicits an inflammatory response characterized by infiltration of mononuclear cells resembling monocytes and lymphocytes from vessels into the intertubular spaces. This response does not commence until Day 4 and occurs at a point just preceding the stage when tubule epithelium shows most evidence of damage (13). Cell infiltration reaches its highest intensity at Day 10, the same time at which the peak of proliferative activity in damaged tubule epithelium occurs. This time sequence suggests that the infiltration of inflammatory cells is mainly a response to the injured tubules, and the restriction of both activities in Zone 1 adds further confirmation.

The major proliferative response elicited by DMN on mesenchyme involves first the interstitial cells of Zones 1 and 2 and later the infiltrating inflammatory cells. Apart from a brief and variable burst of activity involving interstitial cells of Zone 3 from Days 8 to 10, mesenchymal cells of Zones 3, 4, and 5, that is, the pericytes, endothelium, and inner medullary interstitial cells, are not involved in the reaction. Nor in Zone 1 did mesangial cells or any of the cell types associated with the arterioles or juxtaglomerular bodies contribute to the proliferative response. With respect to the brief involvement of Zone 3 interstitial cells, it was not possible by light microscopy to determine whether the labeled cells were lipid granule-containing fibroblasts or agranulocyte-like round cells (1).

The low peak of [3H]Tdr incorporation evident in the interstitial cells of Zone 1 at Day 3 is matched by an equivalent peak of mitosis. Not only are the cells labeled at Day 3 morphologically consistent with fibrocytes (Fig. 5) but, as this increased activity occurs before infiltration of inflammatory cells commences, it implies that the resident interstitial cell of Zone 1 is involved in a relatively synchronous wave of proliferative activity at this stage.

As infiltration of inflammatory cells increases so does the [3H]Tdr incorporation. Although fibrocyte-like cells were also labeled, the major contribution to the high point of proliferative activity at Day 6 seems to come from these...
mononuclear inflammatory cells. Almost certainly, the high peak of labeling at 6 to 7 days involves macrophages as well as lymphocytes. Labeled phagocytes have taken origin presumably in labeled monocytes from the circulation as such cells were involved in margination along blood vessel walls. However, it is also accepted that macrophages can engage in DNA synthesis and subsequent mitotic activity in sites of tissue inflammation (23). The only other mesenchymal component stimulated to proliferate is the capillary endothelium of Zone I, a predictable local event during inflammation. Discrimination between endothelial cells and free interstitial cells was difficult at the stages of obvious cell infiltration, and an error on the side of underscoring endothelial involvement has probably occurred. Even at the electron microscope level, differentiation between resident fibrocytes and endothelium at the height of inflammation is not always possible (10), thus suggesting that the cortical fibrocytes may act as vasoformative reserve elements and become involved in neovascularization.

We have been unable to find any previous reference tracing the proliferative kinetics of interstitial cells of the kidney in response to other nephrotoxins and cannot, therefore, compare the results obtained with DMN. However, the specific action of DMN to initiate a relatively synchronous wave of proliferative activity in a single mesenchymal cell type at Day 3 could be quite relevant to the carcinogenic process. The cell type that appears to be involved, the resident cortical fibrocyte, is also the likely candidate for the derivation of the ultimate mesenchymal tumors as deduced from longitudinal studies of tumor development extending from the time of DMN injection up to the presence of macroscopic neoplasms (9, 10). Furthermore, it is the same cell that appears to be the specific mesenchymal target of the cytotoxic action of DMN. Ultrastructural features of early cytoplasmic injury can be noted in a small proportion of these cells within 12 hr of administration of DMN, 60 mg/kg, although cell necrosis is infrequent (G. C. Hard, unpublished data). If the dose of DMN is raised to a lethal 100 mg/kg, the extent of damage is increased and almost total necrosis of this cell population ensues by Day 3 (10). When the acute 2- to 3-week period of inflammatory response subsides in those rats surviving the liver injury (60-mg/kg DMN model), infrequent hypercellular foci consisting of lymphocytes, lymphoblasts, plasma cells, and macrophages are scattered through the cortex and persist for the next 8 to 10 weeks. These foci are associated invariably with occasional fibroblast-like cells which possess such unusual features as abnormal mitochondria, cell outlines, or nuclei. In the final phase, that of tumor development, the initial foci of interstitial cells that proliferate to form tumors are also fibroblast-like, relating in site of origin and certain cytoplasmic features to the resident fibrocyte of Zones 1 and 2 (10, 11). Consistently then, it is a cell with the same morphological form which shows evidence of specific involvement in each of the various chronological stages of the renal carcinogenic process initiated by DMN.

Autoradiographic analysis of the proliferative activity of renal cell subpopulations, therefore, indicates that a correlation exists between the ability of a carcinogen to cause toxic injury to target cells, to stimulate a pulse of early proliferative activity in the same cell populations, and to induce tumors of a histological type consistent with the cell types involved in the early phase of injury. At the present time there is no adequate explanation for the differential effect of DMN inducing an early proliferative response in interstitial cells at Day 3 but, in contrast, a prolonged effect on epithelium with a peak of activity delayed until Day 10. The degree of necrosis seen in convoluted tubules is probably sufficient for interpretation of the Day 10 peak as a regenerative reaction. However, the infrequency of cell death among resident cortical fibrocytes may preclude regeneration as the sole basis for the wave of early proliferative activity seen in the Zone 1 interstitial cell subpopulation. In speculation, it is possible that it represents the replication phase that may be necessary for acquisition of fixation of the transformation state as suggested by Stewart and Magee (25) in their equivalent biochemical study.

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REFERENCES


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Fig. 1. Section of kidney prepared by perfusion-fixation illustrating the delineation of the 5 zones. Zone 1 is the cortex, Zones 2 and 3 the outer medulla, and Zones 4 and 5 the inner medulla. Paraffin embedding, H & E, × 10.

Fig. 2. Autoradiograph of Zone 1 of rat kidney at 10 days after DMN injection. Two cells in the 2nd segment of a proximal tubule are labeled. Unstained, phase-contrast, × 900.

Fig. 3. Autoradiograph of Zone 1 of rat kidney at 10 days post-DMN. Several profiles of convoluted distal tube are shown. Six of the constituent cells are labeled and 1 (arrowhead) is in mitosis. Unstained, phase-contrast, × 900.

Fig. 4. Autoradiograph of Zone 1 of rat kidney at Day 3 after DMN injection. Five interstitial cells are labeled. Unstained, phase-contrast, × 900.

Fig. 5. Autoradiograph of Zone 1 at Day 3 post-DMN. One interstitial cell shows nuclear label, and a 2nd is in mitosis (arrow). The labeled cell is elongate with well-developed cytoplasm, and the nuclear form is evident. The morphology is typical of a resident cortical fibrocyte. The elongate form and location of the mitotic figure suggest that it is also a resident fibrocyte. Unstained, phase-contrast, × 900.

Fig. 6. Autoradiograph of Zone 1 at Day 6 post-DMN. The interstitium is packed with mononuclear inflammatory cells conforming to macrophages and lymphocytes. Nineteen of the cells show nuclear label. Note the prominent distribution of labeled cells around Bowman’s capsule of glomeruli. G, glomerulus. Unstained, phase-contrast, × 900.

Fig. 7. Autoradiograph of rat kidney at Day 7 post-DMN showing branches of artery and vein at the junction of deep cortex and medulla. The vessels are cuffed with mononuclear inflammatory cells, a significant number of which are labeled. Unstained, phase-contrast, × 900.

Fig. 8. Autoradiograph of rat kidney at Day 7 post-DMN. Despite intravascular perfusion-fixation, the vein lumen in this area of deep cortex shows numerous leukocytes attached to the endothelial lining. Most have the form of monocytes and 2 are labeled. Labeled cells are also present in the surrounding hypercellular interstitial space. Unstained, phase-contrast, × 900.

Fig. 9. Area of deep cortex in an untreated control rat. Intravascular perfusion-fixation has flushed the vessel lumens clear and no leukocytes have been left attached to the endothelial lining. Unstained, phase-contrast, × 375.

Fig. 10. Autoradiograph of Zone 1 at Day 10 post-DMN. The interstitial space in this area is packed with mononuclear inflammatory cells, this being the point when cell infiltration is at its highest. However, only 1 of the cells (arrow) is labeled. The central tubule contains 3 labeled cells. Unstained, phase-contrast, × 900.

Fig. 11. Autoradiograph of Zone 2 at Day 8 post-DMN. Two interstitial cells are labeled but there is no obvious increase in cell numbers in the intertubular space. Note the small vessel near the top in which a few leukocytes show margination. One of these is labeled. Unstained, phase-contrast, × 900.
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