Mitosis and DNA Synthesis in Mouse Kidney: Sources of Error in Evaluating Cell Proliferation[1] (36069)

DANIEL MALAMUD, JEAN PADDOCK, AND RONALD A. MALT
(Introduced by Paul S. Russell)

Surgical Services, Massachusetts General Hospital, and Department of Surgery, Harvard Medical School, Boston, Massachusetts 02114

During studies on cell proliferation in mouse kidney following contralateral nephrectomy, we have noted: (i) a stimulation produced by mild chemical trauma; and (ii) a discrepancy between autoradiographic and biochemical evaluations of DNA synthesis. Comparable phenomena could lead to a misinterpretation of data in other experimental systems.

Materials and Methods. Young adult male Charles River mice (30 g, 42 days) were lightly anesthetized with ether. From some of them, the left kidney, sparing the adrenal, was removed through the flank between 9:00–10:00 a.m., and the pedicle was ligated with 000 chromic catgut. The right kidney [contralateral nephrectomy (CLN)] was harvested later. In other mice (sham operation) the right kidney was lightly touched with a closed forceps, and both kidneys were harvested later. Instruments were cleaned with 70% isopropanol containing 3% hexachlorophene.

For labeling of DNA, ^{3}H (methyl)-thymidine (20 μCi/mouse, 6.7 Ci/mmole; New England Nuclear Corporation) was given by ip injection 30 min before killing. Specific activity of DNA (dpm/mg of DNA) was determined by the method of Scott *et al.* (1) as modified by Hinrichs, *et al.* (2). Samples were counted in Aquafluor scintillator (New England Nuclear Corporation) at 20% efficiency. Autoradiography was done on decapsulated kidneys fixed in 10% buffered formalin, sectioned at 5 μ, dipped in Kodak NTB-2 emulsion, and exposed for 2 weeks (3). Labeled nuclei and mitotic figures were counted in proximal-tubule cells of sectioned kidneys stained with hematoxylin and eosin.

Results. A maximum 2.5-fold increase in DNA specific activity was found 48 hr after CLN (Fig. 1). The right (touched) kidney of the sham-operated animals also showed an increased DNA specific activity. Table I shows that although the ratios of specific activity of the right and left kidney (right/left) of normal animals or of animals etherized only was slightly greater than 1, the ratio (right/left) when the right kidney was touched with forceps was 3.6. When the forceps were wiped completely dry, the ratio was about 1.

Autoradiography of proximal-tubule cells

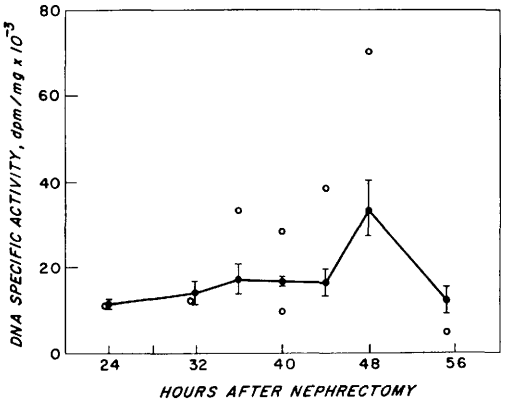

FIG. 1. Specific activity of DNA after CLN: At indicated times after CLN or sham operation, mice were injected with ^{3}H-thymidine and killed 30 min later. DNA specific activity was determined as described in the text. CLN (●) values are mean ± SE for 4 mice. Values for sham-operated animals (○) represent single determinations.

[1] Supported by grants from the Shriners Burns Institute, the NIH (AM-12769), and the American Cancer Society (P-534).

TABLE I. DNA Synthesis in Right and Left Kidney.

Mice were subjected to left nephrectomy, to a sham operation in which the right kidney was touched lightly with a forceps (sham), to ether anesthesia, or they were left untreated. ^{3}H-(methyl)-thymidine (20 μCi) was injected at 47.5 hr after laparotomy and mice killed 30 min later. DNA specific activity was determined as described in text. Numbers of mice in parentheses. Values indicate mean ± SE.

Treatment	DNA specific activity (dpm/mg of DNA)		
	Left kidney	Right kidney	Right/left
None (5)	11,989 ± 2087	13,088 ± 2327	1.1
Ether only (5)	8760 ± 1396	10,042 ± 1542	1.2
Sham (6)	6100 ± 1299	22,059 ± 7248	3.6
Left nephrectomy (6)		23,522 ± 4090	

confirmed the increase in ^{3}H-thymidine incorporation produced 48 hr after CLN (Fig. 2). A mitotic wave was observed at about the same time (Fig. 2). The distribution of radioactively labeled cells was strikingly different, however, between CLN kidneys and the touched kidneys of shams. In CLN kidneys, labeled cells were distributed throughout the proximal-tubules (Fig. 3A). In touched kidneys, labeled cells were confined to the perimeter of the kidney in the area that was touched; furthermore nonparenchymal cells were labeled as well as tubular cells (Fig. 3B).

Discussion. The increase in specific activity of DNA in mouse kidneys at 48 hr after CLN was compatible with the increased labeling index and mitotic response in these organs after CLN, and was similar to previous results obtained in rats (4, 5). Although assessments of the exact magnitude of stimulated cell proliferation after CLN have differed (5–7), there may be no real discrepancies, since some assessments have been of the mitotic index and others have been of an aspect of DNA synthesis.

The unexpected finding in the present study was the intense labeling seen at the periphery of the kidney touched with forceps imperceptibly wet with isopropanol. This minimal trauma caused a striking response involving both parenchymal and nonparenchymal cells in the subcapsular area of the kidney. This type of proliferation is distinct from that occurring after major injury to the kidney, in which the proliferative response appears both in the affected and in the contralateral kidney (8). The magnitude of the response reported here was probably accentuated by the ip route of injection of ^{3}H-thymidine; heavy subcapsular labeling of DNA has previously been observed in lightly

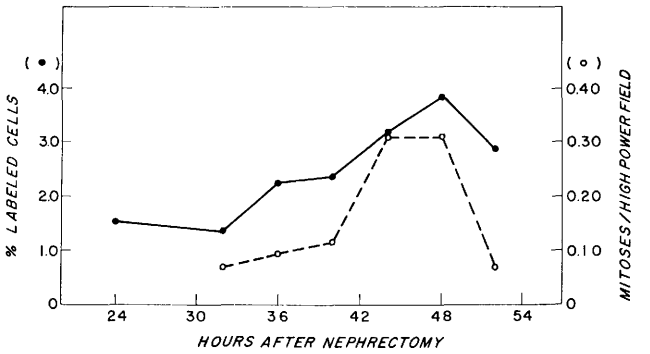

FIG. 2. Labeling and mitotic indices after CLN: Percentage of labeled cells (●) was determined by counting a minimum of 1000 proximal-tubule cells. Mitoses/high power field (○) represents the average for 25 fields.

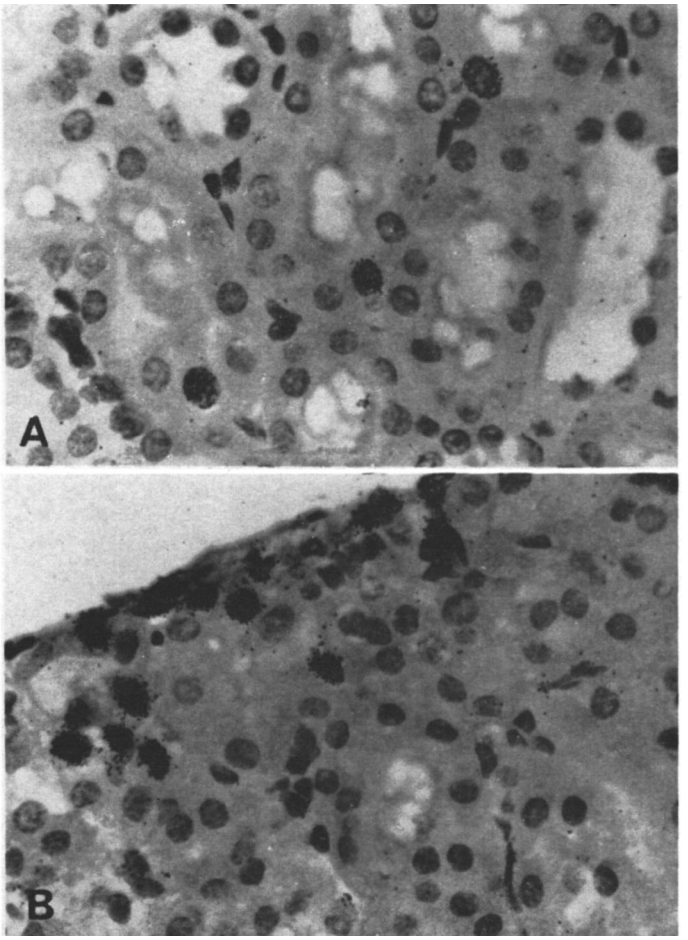

FIG. 3A. Autoradiograph of kidney 48 hr after CLN: Note labeled proximal-tubule cells; magnification 400×. (B) Autoradiograph of kidney 48 hr after touching with forceps: Note intense labeling at periphery of tissue; magnification 400×.

handled rat liver after ip injection of ^{3}H-thymidine, but not after iv injection (9).

Although the data in Fig. 1 and Table I might at first suggest that touching of a kidney is as effective a stimulus for cell proliferation as CLN, autoradiographic analysis showed that the alcohol–hexachlorophene on the forceps led to the stimulation of a small population of subcapsular cells incorporating a relatively large amount of isotope. There was no generalized proliferative response of proximal-tubule cells. Similar artifacts resulting from minimal trauma could perhaps also be produced after injections of tissue homogenates or serum or by manipulations within the peritoneal cavity. The mitotic index, the labeling index, and the specific activity of DNA cannot be used interchangeably in assessing cell proliferation in kidney.

Summary. Approximately 48 hr after removal of one mouse kidney, the specific activity of DNA increased about 2-fold and was confirmed by autoradiographic studies and mitotic counts. A similar increase in DNA specific activity was produced by touching a normal kidney with forceps imperceptibly wet with isopropanol–hexachlorophene, but autoradiography showed proliferative activity confined to the subcapsular region rather than throughout the proximal tubules.

1. Scott, J. F., Fraccastoro, A. P., and Taft, E. B., J. Histochem. Cytochem. **4**, 1 (1956).

2. Hinrichs, H. R., Petersen, R. O., and Baserga, R., Arch. Pathol. **78**, 245 (1964).

3. Baserga, R., and Malamud, D., "Autoradiography: Techniques and Applications." Harper & Row (Hoeber), New York (1969).

4. Johnson, H. A., and Vera Roman, J. M., Amer. J. Pathol. **49**, 1 (1966).

5. Phillips, T. L., and Leong, G. F., Cancer Res. **27**, 286 (1967).

6. Williams, G. E. G., Brit. J. Exp. Pathol. **42**, 386 (1961).

7. Reiter, R. J., Lab. Invest. **14**, 1636 (1965).

8. Argyris, T. S., and Trimble, M. E., Anat. Rec. **150**, 1 (1964).

9. Weinbren, K., Arden, G. M., and Stirling, G. A., Brit. J. Exp. Pathol. **50**, 540 (1969).

Received July 15, 1971. P.S.E.B.M., 1972, Vol. 139.