Cell Proliferation in Rat Kidney Induced by Lead Acetate and Effects of Uninephrectomy on the Proliferation

David D. Choie, MS and Goetz W. Richter, MD

Effects of a single dose of lead (0.04 mg lead g body weight) on the proliferation of proximal tubular epithelium in rat kidneys were investigated by autoradiography over a period of 72 hours, using ^{3}H-thymidine as a label. The results demonstrate that cell proliferation was greatly stimulated within 2 days after lead was injected. The increase in DNA synthesis began about 20 hours after intraperitoneal injection of lead, reached a sharp peak at 30 hours, and declined rapidly thereafter. At the peak, the mean labeling activity was 40 times that observed in control rats. Cumulatively, an average of 14.5% of the proximal tubular epithelial cells were labeled 72 hours after lead was injected. When uninephrectomy was followed immediately by injection of lead, the stimulation of DNA synthesis in the remaining kidney was, on the average, greater than the sum of the separate effects of the two treatments. This indicates that the stimulatory effects of uninephrectomy and injection of lead on renal cell proliferation were additive. (Am J Pathol 66:265–276, 1972)

LITTLE IS KNOWN about the early phases of effects of lead on cellular metabolism, although interference with enzymes involved in heme biosynthesis has been well documented.[1] Chronic intoxication with lead in experimental animals results in structural changes in renal tubular epithelial cells—notably disruption of mitochondria [2,3] and formation of intranuclear inclusion bodies.[4-7] Lead also has carcinogenic effects in kidneys of rats and mice after prolonged intoxication.[8-10] For these and other reasons, it became desirable to investigate the effects of lead on cell proliferation in mammalian kidneys.

Results of experiments to be reported in this paper indicate that in rat kidneys replication of proximal tubular cells, as shown by autoradiography, is markedly stimulated within 2 days after intraperitoneal injection of a single dose of lead acetate. The time sequence of cell proliferation induced by lead was found to differ from that in compensatory renal hyperplasia after unilateral nephrectomy. In rats subjected to both uninephrectomy and injection of lead, proliferation of proximal tubular cells was markedly enhanced.

From the Department of Pathology, The University of Rochester Medical Center, Rochester, NY 14642.

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Address reprint requests to Dr. Richter.

Materials and Methods

Adult, female, Sprague-Dawley, albino rats weighing 220–270 g were used (Chordata Corp, Rochester, NY). They were caged individually, and given Purina rat chow and tap water *ad libitum*. The animal room was air-conditioned at 22 C, and maintained on 12-hour cycles of light and dark.

Four experimental groups were set up.

1. Controls: untreated.
2. Uninephrectomized group: left kidney removed at hour zero through dorsolateral incision under semi-aseptic conditions.
3. Leaded group: a single dose of lead acetate in sterile water, 0.04 mg lead/g body weight, injected intraperitoneally at hour zero.
4. Uninephrectomized, leaded group: left nephrectomy as in group 2 at hour zero, and injection of a single dose of lead as in group 3 within 20 minutes after the operation.

All experiments were initiated between 10 and 12 AM. In pulse-labeling experiments, ^{3}H-thymidine (specific activity 15.2 Ci/mmole, diluted with distilled water to 100 µCi/ml; Schwarz/Mann, Orangeburg, New York) was injected intraperitoneally in doses of 0.2 µCi/g body weight 1 hour before sacrifice. In "continuous labeling," ^{3}H-thymidine was injected intraperitoneally in doses of 0.12 µCi/g body weight every 8 hours from the beginning of the experiment until the time of sacrifice.

Animals were sacrificed with ether in groups of three at each time point. Each kidney was weighed and split from pole to pole into anterior and posterior portions, and a slice, 2 mm in thickness, was fixed in Carnoy's fluid, embedded in paraffin, then sectioned at 4 µ. Autoradiographs were prepared from sections by dipping into NTB2 nuclear track emulsion (Eastman Kodak Co, Rochester, New York). The coating method utilized has been described by Baserga and Malamud.[11] The slides were exposed for 2 weeks at 4 C, and developed in Kodak D-9 developer, fixed, washed, and stained with hematoxylin and eosin. In each autoradiograph, proximal tubular cells of the outer cortex were scored to determine labeling and mitotic indices. Up to 10,000 cells were scored for each animal, using an oil-immersion objective.

Results

No animals died during the experimental period of 72 hours. Among the uninephrectomized rats, the wet weights of the remaining (right) kidneys were slightly higher (~10%) 72 hours after uninephrectomy than those of right kidneys from controls. The difference, however, was not statistically significant. An increase in wet weight was also noted in the right kidneys of rats subjected to left uninephrectomy and injection of lead. Among the rats treated with lead alone, there was little change in wet weights of kidneys after 72 hours. Histologically, tubular cells appeared normal, and no necrosis or exfoliation of tubular epithelial cells was detected in kidneys of rats treated with lead.

Labeling indices are presented as the number of cells labeled per 1000 cells in the proximal tubular epithelium, and mitotic indices as the number of mitoses per 1000 cells scored. Results are expressed as arithmetic means (± SE) for the 3 animals at each time point.

Control Group

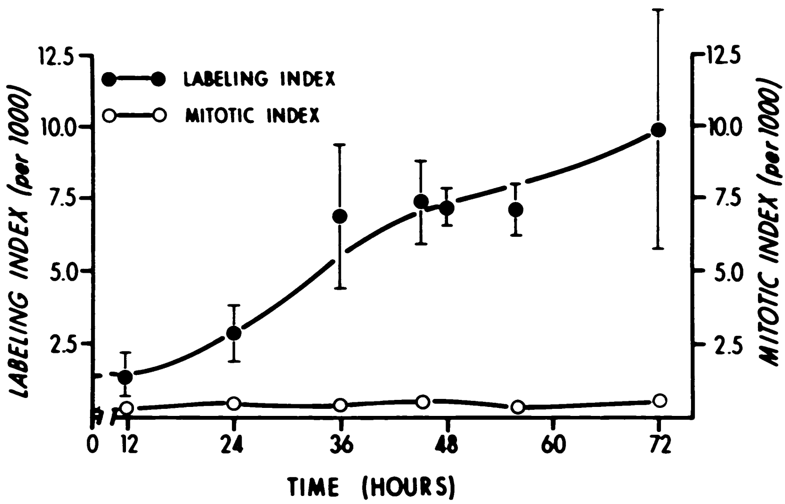
The mean labeling index in pulse-labeled controls was 1.5/1000 cells. This value did not change significantly during the 72-hour experimental period. Continuous labeling resulted in accumulation of labeled cells. The mean cumulative labeling index after 72 hours was 10/1000 cells in the proximal tubules of the control animals (Text-fig 1).

Effects of Uninephrectomy on the Remaining Kidney

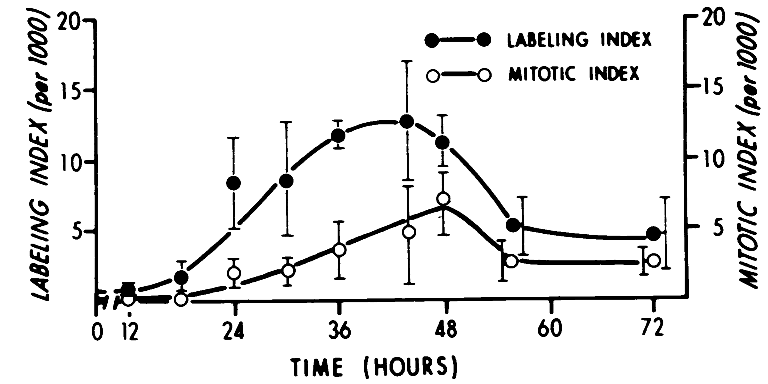
In the pulse-labeling experiments, labeling activity in the remaining kidney began to increase around 20 hours after uninephrectomy, and reached a peak around 44 hours (Text-fig 2). At the peak, the mean labeling index was 12.3/1000 cells, which was about 8 times that for the controls ($P < 0.05$). Mitotic indices reached a peak at 48 hours. After continuous labeling, the mean cumulative labeling index was 44/1000 cells in the remaining kidney, 72 hours after uninephrectomy (Text-fig 3).

Effects of a Single dose of Lead on Cell Proliferation in Rat Kidneys

In rats given a single dose of lead acetate, DNA synthesis was significantly stimulated in both kidneys. In the pulse-labeling experiments, labeling activity started to increase around 20 hours after the lead was injected, and reached a peak at 30 hours (Text-fig 4). At the peak, the



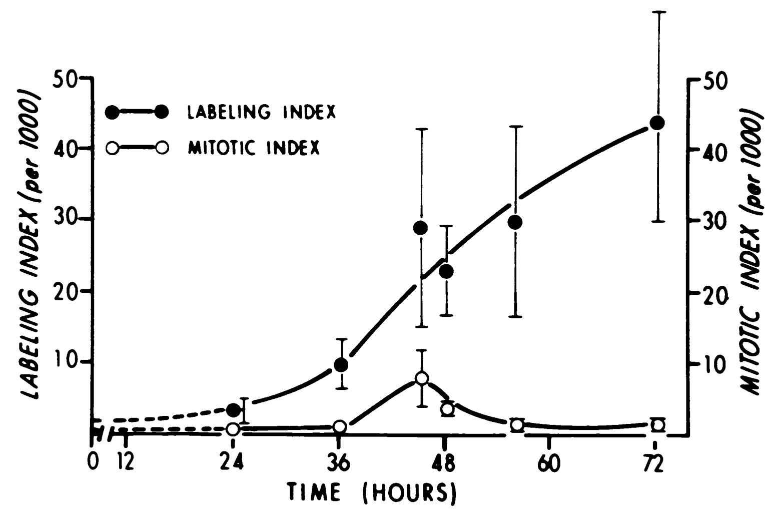
TEXT-FIG 1—Untreated controls. Labeling and mitotic indices in the proximal tubular epithelium of kidneys of rats injected with ^3H-thymidine every 8 hours. In this and in the subsequent text-figures, each point represents the mean scores of 3 rats.



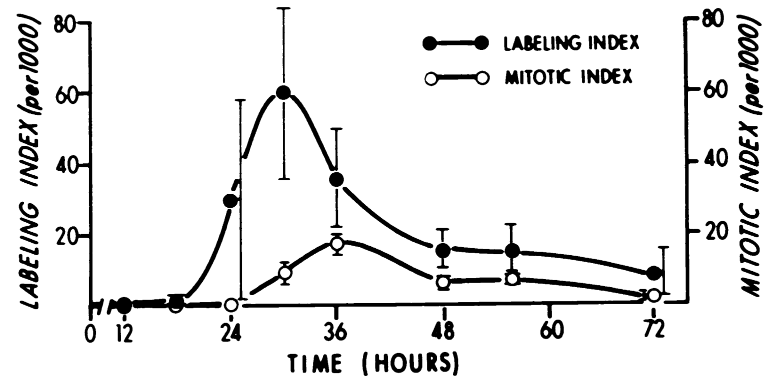
TEXT-FIG 2—Effects of uninephrectomy on labeling and mitotic activities in the proximal tubular epithelium of the remaining kidney. Animals were uninephrectomized at zero-hour, and injected with ^{3}H-thymidine 1 hour before sacrifice.

mean labeling index was 60.5/1000 cells, which was about 40 times greater than the control value ($P < 0.05$). The labeling activity decreased rapidly after 30 hours, but was still higher than in controls at 72 hours.

In the pulse-labeling experiments, cells in mitosis were not labeled, which indicates that the G_2 phase lasted longer than 1 hour—the duration of labeling. The mitotic indices reached a peak 36 hours after lead was injected. This peak of mitotic activity lagged behind that of label-



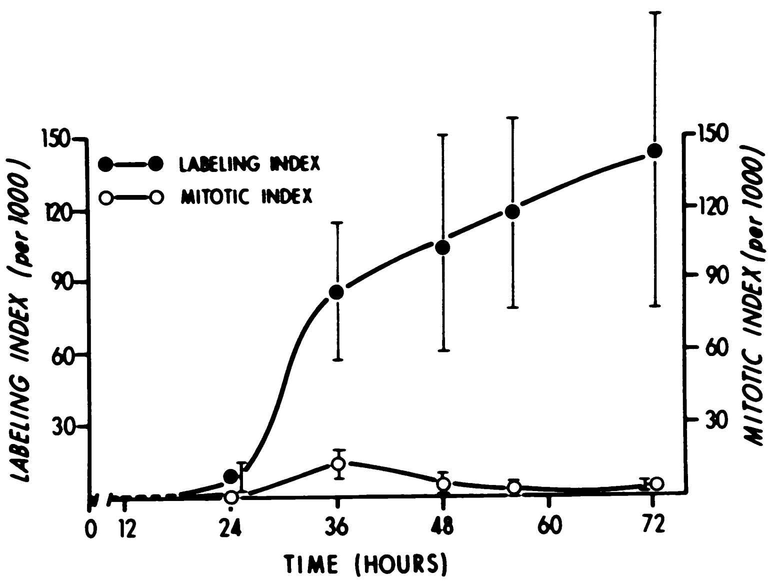
TEXT-FIG 3—Cumulative labeling and mitotic indices in the remaining kidney after uninephrectomy. Animals were injected with ^{3}H-thymidine every 8 hours.



Text-fig 4—Effects of a single dose of lead on labeling and mitotic activities in the proximal tubular epithelium of kidneys of rats injected with lead (0.04 mg/g body wt) at zero-hour. ^{3}H-thymidine was injected 1 hour before sacrifice.

ing activity by about 6 hours. The lag of mitotic activity indicates that about 6 hours were required for the cells to proceed from the midpoint of the S phase to that of the M phase in the kidneys of rats treated with lead.

In the continuous-labeling experiment, the cumulative labeling indices increased rapidly between 24 and 36 hours after injection of lead (Text-fig 5). This coincided with the period of maximum labeling

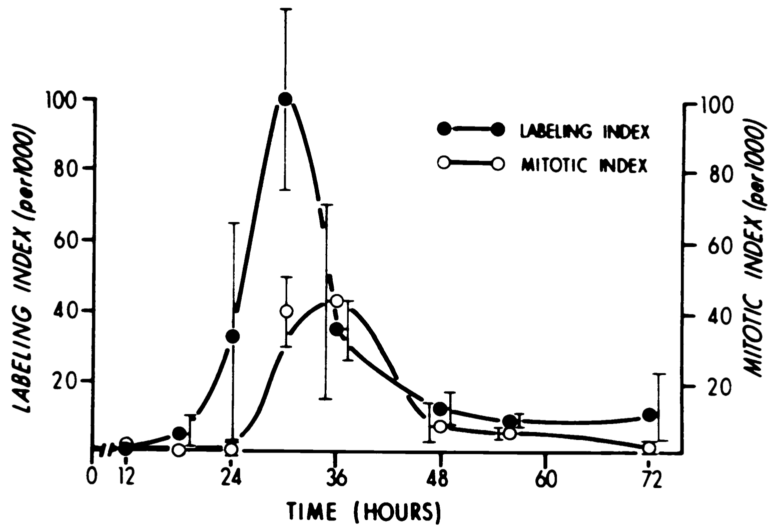


Text-fig 5—Cumulative labeling and mitotic indices in the kidneys after injection of lead at zero-hour. Animals were injected with ^{3}H-thymidine every 8 hours.

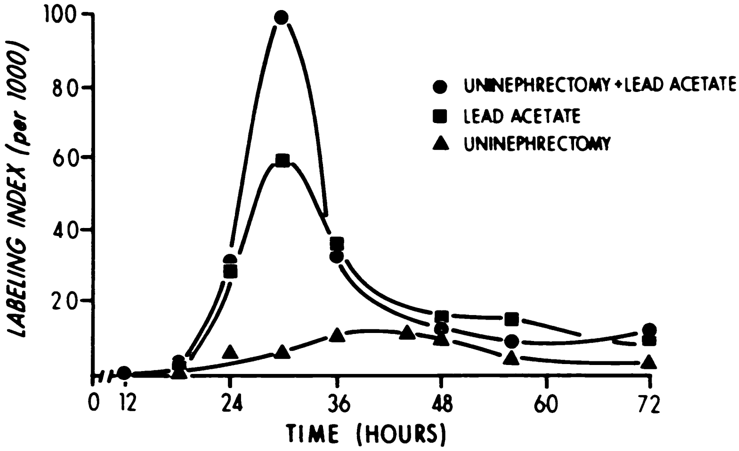
activity seen in the pulse-labeling experiment. The mean cumulative labeling index at 72 hours was 145/1000 cells, which was about 15 times above the control level ($P < 0.05$). As expected, cells in mitosis were labeled in the continuous labeling. Mitotic indices reached a peak around 36 hours, in agreement with the findings in the pulse-labeling experiment. Although only the epithelial cells of proximal convoluted tubules were scored, labeled cells were present in all parts of nephrons in rats treated with lead.

Effects of Uninephrectomy plus Injection of Lead on Cell Proliferation in Rat Kidneys

The dual treatment of uninephrectomy and injection of lead stimulated DNA synthesis in the remaining kidney most significantly. In the pulse-labeling experiment, the labeling activity started to increase after about 20 hours, reached a peak at 30 hours, and declined rapidly thereafter (Text-fig 6). At the maximum point, the mean labeling index was 100/1000 cells, which was about 65 times the control value ($P < 0.01$). In pulse-labeling experiments, the patterns of labeling activity were strikingly similar in the leaded group and in the uninephrectomized leaded group. This is shown in a composite graph in Text-fig 7. In both groups, proliferative activities are characterized by a single sharp increase between 24 and 36 hours, with maximum labeling activities super-



Text-fig 6—Effects of uninephrectomy plus injection of lead (0.04 mg/g body wt) on labeling and mitotic activities in the remaining kidney. Animals were uninephrectomized and injected with lead at zero-hour. ^{3}H-thymidine was injected 1 hour before sacrifice.



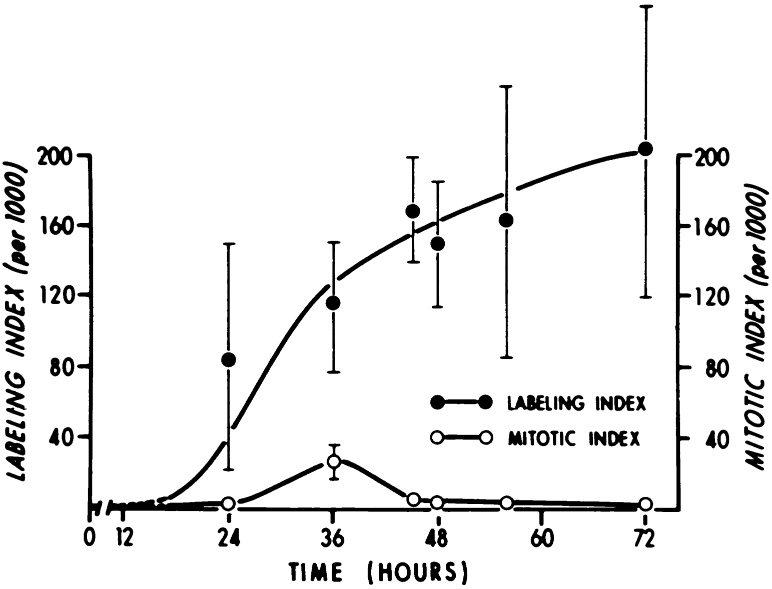
TEXT-FIG 7—Composite graph of labeling indices in the kidneys of three experimental groups in pulse-labeling experiments.

imposed at 30 hours. The dual treatment stimulated greater cellular proliferation in proximal tubular epithelium than did uninephrectomy or treatment with lead alone. In fact, the degree of stimulation achieved with the dual treatment was greater than the sum of the effects of the two separate stimuli (Text-fig 7). Apparently, the combined stimulatory effects of lead and uninephrectomy on tubular cell proliferation are additive.

After continuous labeling for 72 hours, the mean labeling index was 20.8% in the proximal tubular epithelium in kidneys of rats subjected to the dual treatment (Text-fig 8). This value is over 20 times the control level ($P < 0.05$). Cells in mitosis were labeled, and mitotic activity was at a peak at 36 hours. Text-fig 9 shows the results of all continuous-labeling experiments.

Discussion

The results of these experiments demonstrate that a single dose of lead acetate significantly stimulates cell proliferation in the proximal tubular epithelium of rat kidneys. The well-defined burst of DNA synthetic activity around 30 hours indicates that, after an injection of lead, the stimulated cells are highly synchronized in their transit from the presynthetic (G_1) to the synthetic (S) phase. In contrast, no synchronization in flow of cells was observed in kidneys of uninephrectomized rats. It is noteworthy, however, that the initial increase in labeling activity occurred about 20 hours after uninephrectomy, injection of lead or combination of the two treatments. It is interesting that the stimu-

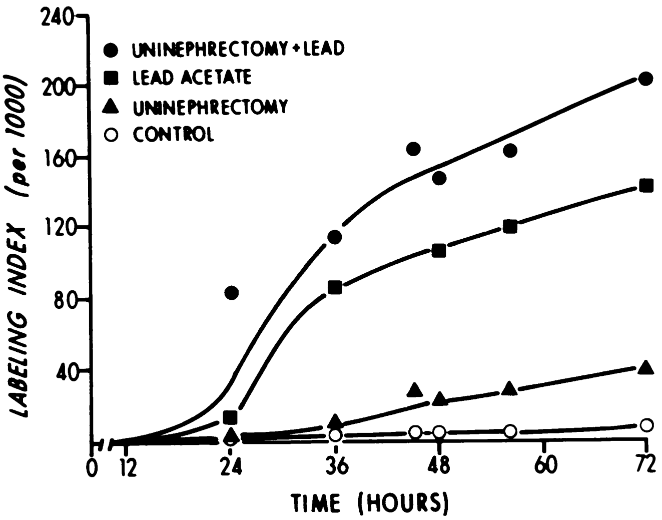


Text-fig 8—Cumulative labeling and mitotic indices in the remaining kidney after uninephrectomy plus injection of lead at zero-hour. Animals were injected with ^{3}H-thymidine every 8 hours.

latory effects of uninephrectomy and treatment with lead on cell proliferation are additive, and that the maximum labeling activity takes place 30 hours after dual treatment as it does in rats treated with lead alone.

The adult mammalian kidney normally maintains a low mitotic activity, but renal cell proliferation can be stimulated in various ways. Uninephrectomy is known to produce hyperplasia and hypertrophy in the remaining kidney. [12,13] Ligation of one urether [14,15] metabolic acidosis,[16] renal trauma,[17,18] exposure to cold,[19] high NaCl in the drinking water,[15] K^+ deficiency in the diet [20] and a high gelatin diet [21] have been reported to stimulate moderate renal cell proliferation in experimental animals. Folic acid has been shown to stimulate a marked increase in DNA synthesis in kidneys of rats and mice.[22,23]

Two hypotheses have been proposed to explain the mechanism of renal cell proliferation in compensatory hyperplasia. One hypothesis postulates that a reduction of the kidney mass produces humoral factors—either an increase in stimulators [24,25] or a decrease in inhibitors.[26] Another hypothesis is that increased reabsorptive work load stimulates renal hyperplasia.[12,27] However, both of these hypotheses have been challenged.[17,18,28,29]



TEXT-FIG 9—Composite graph of cumulative labeling indices in the kidneys of four experimental groups in continuous labeling experiments.

The mechanism of the cellular proliferation induced by lead remains to be elucidated. It is not a regenerative response because the tubular epithelium was unaltered after lead was injected, and because there was no detectable loss of tubular cells during the experimental period. In the absence of reduction of kidney mass, humoral factors are unlikely to have been involved. Nor would increase in the work load explain the cell proliferation induced by lead, since others have found no significant change in glomerular filtration rate in rats treated with lead.[30] On the other hand, the synchronous burst of DNA synthesis after treatment with lead suggests that lead might be directly involved in triggering DNA replication.

The onset of DNA synthesis is thought to require synthesis of RNA and proteins.[31,32] There have been reports that synthesis of RNA and protein occurs during the 20-hour lag period after uninephrectomy.[12] In the present experiments, injection of lead was followed by a quiescent period of about 20 hours before the onset of increased activity in DNA synthesis. This suggests that, during the lag period, synthesis of RNA and protein may have taken place in the kidneys of rats treated with lead. It is possible that lead triggers synthesis of DNA by inactivating inhibitory substances which regulate synthesis of specific RNA and proteins that are prerequisite for synthesis of DNA.

Since lead is readily identifiable by various means, it may prove to be useful for probing the trigger mechanism of DNA synthesis in mammalian cells. More detailed information about the phenomenon reported here is necessary before definite conclusions can be drawn. The possibility that stimulation of cell proliferation induced by lead may be related to the carcinogenic effects of lead also deserves further investigation.

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