Stimulation of DNA Synthesis in Rat Kidney by Repeated Administration of Lead[1] (37043)

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A single dose of lead stimulates a wave of nuclear DNA synthesis and cell proliferation in the tubular epithelium of rat kidneys within 2 days (1). The proliferative wave occurs during a discrete period beginning approximately 20 hr and reaching a peak 30 hr after an intraperitoneal injection of lead. It has also been shown that incorporation of ^{3}H-thymidine by tubular epithelium in rat kidneys is increased in chronic lead intoxication, after many intraperitoneal injections of lead, given over a period of 6 mo (2). We have extended these studies to investigate whether administration of lead immediately following the initial wave of nuclear DNA synthesis would excite a new wave in renal tubular epithelium. We have found, by means of autoradiography, that intraperitoneal injections of lead, repeated three times in 2-day intervals, stimulated successive waves of nuclear DNA synthesis in the tubular epithelium of rat kidneys.

Materials and Methods. Adult, female, Sprague-Dawley albino rats, weighing 200–250 g were used (Chordata Corp., Rochester, NY). They were housed individually in a room with 12-hr cycles of light and dark, and were given Purina rat chow and tap water *ad libitum*. Rats were divided into three experimental groups and one control group. The first experimental group was injected with lead acetate in sterile water in doses of 0.05 mg lead/g body weight, intraperitoneally, at 0 hr. The second group was similarly injected with lead at 0 hr and after 48 hr, and the third group at 0 hr, after 48, and 96 hr. Controls were injected with sodium acetate solution (0.06 mg Na acetate/g body wt) at 0 hr. At specified intervals during a 7-day period, groups of 3 rats were sacrificed with ether (Fig. 1). On hour prior to sacrifice, all rats were injected intraperitoneally with ^{3}H-thymidine (sp act 6.0 Ci/mmole, Schwarz/Mann, Orangeburg, N.Y.) in doses of 0.5 μCi/g body weight.

Each kidney was excised and cut twice through the long axis so as to yield a central slice. The tissues were fixed in Carnoy's fluid, embedded in paraffin, sectioned at 5 μm, and mounted on glass slides. The renal tissues were autoradiographed by dipping the slides into NTB-2 nuclear track emulsion (Eastman Kodak Co., Rochester, NY). The slides were exposed for 2 wk, developed in D-19 Kodak developer, fixed, washed, and stained with hematoxylin and eosin.

Labeling and mitotic indices were determined for the proximal and distal tubular epithelium in the renal cortex. For each animal, at least 100 microscopic fields were examined with an oil immersion objective. In leaded rats and in controls, there were 72 $\pm$ 11 (SD) tubular epithelial cells/microscopic field.

Results. 1. Effects of a single dose of lead on incorporation of ^{3}H-thymidine into nuclear DNA of tubular epithelial cells. A single injection of lead stimulated a wave of DNA synthesis in the proximal and distal tubular epithelium (Fig. 1a). Initially, there was a lag period of approximately 20 hr, during which uptake of ^{3}H-thymidine remained unchanged. After this period, the labeling activity increased rapidly, and reached a peak approximately 30 hr after the injection, then declined to a residual level that lasted 5 days. At the 30-hr point the maximum mean labeling index was 145 per 50 micro-

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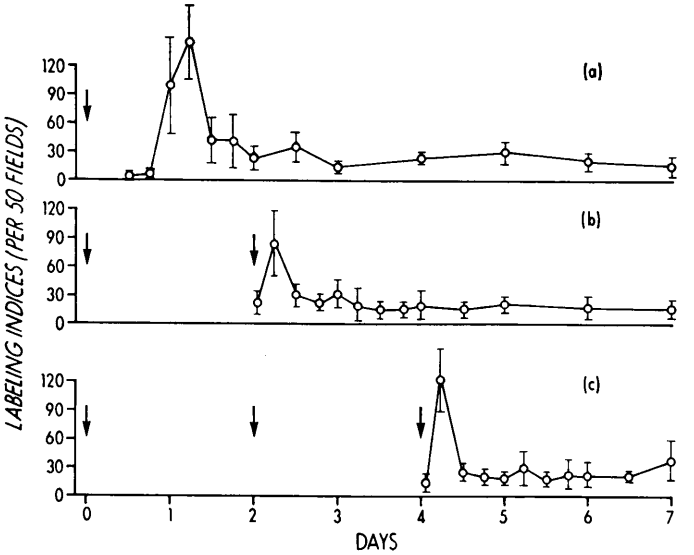

FIG. 1. Labeling indices in nuclei of epithelial cells of proximal and distal tubules in rat kidneys after one (a), two (b), and three (c) injections of lead (0.05 mg lead/g body wt). Renal cells were pulse-labeled with ^{3}H-thymidine for 1 hr. Each point represents the mean number of labeled cells ± SD per 50 microscopic fields from each of 3 rats. Injections of lead are indicated by arrows. Mean labeling index of controls, given sodium acetate (0.06 mg/g body wt), was 4/50 fields.

scopic fields, or approximately 40 times the control level. The residual labeling index after the wave, 25 per 50 microscopic fields, though considerably less than the maximum, was still above the control level, which was 4 per 50 microscopic fields. There was an increase in mitotic indices in the tubular epithelium following the rise of labeling activity. Control kidneys showed no significant changes in labeling indices during the 7-day period.

2. Effects of a second dose of lead. A second injection of lead, given 48 hr after the first, excited a second wave of DNA synthesis in the renal tubular epithelium (Fig. 1b). Peak labeling activity was observed 6 hr after the second dose of lead, in contrast to the 30-hr interval after the first injection. Although the peak of the second wave was lower than that of the first, it was significantly ($p < 0.02$) greater than the labeling activity at the comparable time point after a single dose of lead (Fig. 1a and b). Following the second wave of cellular proliferation, uptake of ^{3}H-thymidine into nuclei of proximal and distal tubular epithelial cells remained at a residual level comparable to that seen after a wave of DNA synthesis induced by a single dose of lead.

3. Effects of a third dose of lead. A third injection of lead, administered 48 hr after the second, and 96 hr after the first, produced a third wave of cellular proliferation in the proximal and distal tubular epithelium (Fig. 1c). The third wave occurred as swiftly as the second wave. The maximum mean labeling index (122 per 50 microscopic fields) 6 hr after the third dose was significantly ($p < 0.01$) greater than the labeling indices at the comparable time points after one or two doses of lead (Fig. 1a, b, and c). Following the third wave, labeling activity remained at a residual level, which was approximately 6 times above that in controls.

Discussion. As shown in Fig. 1, 1 to 3 injections of lead stimulated successive waves of DNA synthesis in the epithelium of proximal and distal tubules of rat kidneys. The magnitude of the peak labeling activity was somewhat greater in the first wave than in the second or third, but the difference was not significant ($p > 0.1$). The increase in

labeling activity took place much sooner after the second and third dose of lead than after the first. The amount of lead absorbed was apparently less than the total amount of lead injected because lead precipitate was seen in the peritoneum at the time of sacrifice.

The tubular epithelium of adult rat and mouse kidneys normally maintains a low mitotic activity in all segments (1). However, a variety of experimental stimuli can produce cellular proliferation in the renal tubules, for example, contralateral nephrectomy (3, 4), renal trauma (5), metabolic acidosis (6), or administration of folic acid (7, 8). We have reported previously that a single dose of lead (0.04 mg/g body wt) stimulates cellular proliferation in the proximal and distal tubular epithelium in rat kidneys (1), and have also reported that DNA synthesis is significantly elevated in renal tubular epithelium in rats after chronic treatment with lead (2). It is known that in rats and mice treatment with lead during 1 yr results in adenomas and carcinomas of the kidneys (9, 10). The increase in cellular proliferation in the kidney during acute and chronic intoxication with lead suggests that the carcinogenic effect is related to prolonged stimulation of nuclear DNA replication in tubular epithelial cells over long periods. Indeed, one of the biological characteristics of carcinogenesis is an increase in cellular proliferation in the target tissue during the so-called latent period (11, 12).

The nature of the stimulus of DNA synthesis induced by a single and by multiple doses of lead is unknown. The tubular epithelial cells that were stimulated apparently proceeded to cell division because increased labeling indices were followed by increased mitotic indices. Interestingly, each wave of DNA synthesis lasted only for a short time after each dose of lead. The waves of stimulated DNA synthesis were too brief to be accounted for entirely by elimination of lead from renal tissue, which takes days (13). Possibly, the mitogenic effect of lead in the kidney may be quickly curtailed by an active cellular response to lead toxicity. It has been proposed that in lead poisoning the intranuclear inclusions that develop in the epithelial cells of the proximal tubules protect the cells against toxic effects of lead by trapping intracellular lead (14). We have reported that in rat kidneys intranuclear inclusions appear as early as 1 day after a single injection of lead (15). The rapid formation of intranuclear inclusions may prohibit or minimize lead-induced DNA replication by reducing the amount of "free" intracellular lead in the epithelial cells of proximal tubules. However, the lack of such inclusions in the *distal* tubules—which also show increased DNA synthesis—needs further explanation. The cellular proliferation induced by lead is not likely to be a regenerative response because there was no evidence of tubular necrosis. Yet, if cellular proliferation remains increased (or is often increased) during long periods, it is unclear why the kidneys do not increase much in size (2). One possible reason is that lead may accelerate the turnover rate of the epithelial cells. If increased cellular proliferation is in balance with enhanced cell turnover (*i.e.*, shedding of epithelial cells into the tubules, followed by their replacement with new cells), the total number of cells in the renal tubules may change little.

The stimulatory effect of lead on nuclear DNA synthesis in rat kidneys may seem paradoxical considering the inhibitory actions of lead, such as disturbances in the biosynthesis of heme (16). However, it is possible that lead interferes with the mechanism that regulates replication of DNA so as to unleash cellular multiplication.

In chronic lead poisoning, alterations in the regulation of DNA synthesis that result in increased cell proliferation during prolonged periods might increase the chances that irreversible changes linked to carcinogenesis take place.

Summary. Effects of repeated administration of lead on DNA synthesis in rat kidneys were investigated by autoradiographic methods. In the epithelium of proximal and distal tubules, three injections of lead (0.05 mg lead/g body wt), given at 2-day intervals, excited successive waves of cellular DNA synthesis, 20–40 times above the normal level. The peak of the initial wave of DNA synthesis occurred 30 hr after the first dose

of lead, whereas the peaks of the second and third waves appeared 6 hr after the second and third doses of lead, respectively. After each wave of ^{3}H-thymidine uptake, labeling activity declined to a level that remained approximately 6 times that in control rats.

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