Lead and Liver Cell Proliferation

Effect of Repeated Administrations

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The effect of repeated treatments with lead on hepatic cell proliferation was investigated in male Wistar rats. The animals were given intravenous injections of lead nitrate once every 10 days for 30 and 80 days. At the end of the experimental regimen, enlargement of the liver, accompanied by an increase in hepatic DNA content, was observed. A significant enhancement in the incorporation of labeled thymidine into hepatic DNA was found in lead-treated rats at the time intervals mentioned above, when compared with controls. An increase in the number of liver cells involved in mitosis was also observed in lead-treated animals. Analysis of serum glutamic-pyruvic transaminase and histologic observations did not show any sign of cell death at the time points examined. These results indicate that liver cells exposed to repeated treatments with lead undergo proliferation. However, a progressive reduction in the capacity of hepatic cells to divide was found in rats given repeated administrations of the metal, when compared with the extent of cell proliferation induced by a single dose of lead nitrate. (Am J Pathol 1983, 113: 315–320)

EXPOSURE to lead, particularly through its wide commercial use, results in a variety of lesions. In addition to disturbances of the central nervous system,[1] impairment of hematopoiesis,[2] and cardiovascular damage,[3] lead has been shown to induce biochemical alterations in the liver and kidney.[4–6] Choie and Richter have reported that one of the consequences associated with acute lead exposure is a stimulation in the biosynthesis of nucleic acids in mouse as well as in rat kidney.[7–8] An increased proliferation of proximal tubular epithelium cells, along with the appearance of intranuclear inclusion bodies, was also found in kidneys of rats treated with lead for 6 months.[9]

Despite the large accumulation of lead in the liver,[10] an area of lead toxicity that has received little attention is the influence of the metal on hepatic nucleic acid synthesis.

Supported by funds from the Consiglio Nazionale delle Ricerche, Roma, within the special project Medicina Preventiva e Riabilitativa, and from Assessorato Igiene e Sanità, Regione Autonoma della Sardegna, Cagliari, Italy.

Accepted for publication June 20, 1983.

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We have recently reported the mitogenic effect on the liver of acute exposure to lead nitrate.[11]

The purpose of the present investigation was to determine if the same stimulatory effect on liver cell proliferation could be achieved by a prolonged treatment with lead.

The results indicate that repeated intravenous injections of lead nitrate induce cell proliferation, as shown by the incorporation of labeled thymidine into DNA and the mitotic index, even if a decrease in the extent of nucleic acid synthesis was found in rats treated for 30 and 80 days, when compared with animals exposed to a single dose of the metal.

Histologic and biochemical observations did not show any sign of cell death.

Materials and Methods

Animals

Male Wistar rats (180–200 g) fed a semisynthetic diet were maintained on a daily cycle of alternating 12-hour periods of light and darkness. In all experiments the animals had free access to food and water.

Experimental Groups

Group 1 rats were injected with a single dose of lead nitrate.

Group 2 rats were given four injections of lead once every 10 days, for 30 days.

Group 3 rats were given nine injections of lead, once every 10 days, for 80 days.

Control animals were injected intravenously with distilled water; subgroups of 6–8 animals were sacrificed concomitantly with the experimental rats. All the animals were killed 36 hours after the last injection.

Chemicals

Lead nitrate (Carlo Erba, Milano, Italy) dissolved in distilled water was injected intravenously at a dose of 5 μmol/100 g body weight under light ether anesthesia. ^{3}H-Thymidine was purchased from The Radiochemical Centre, Amersham, United Kingdom.

Determination of Hepatic DNA Content

After sacrifice the livers were frozen at −70 C. Total hepatic DNA was quantitatively assayed by Burton's diphenylamine method.[12]

Determination of Specific Activity of DNA

Following the last administration of lead nitrate the animals were given an intraperitoneal injection of ^{3}H-thymidine (specific activity 25 Ci/mmol) at a dose of 50 μCi/100 g body weight, 1 hour before sacrifice.

The hepatic chromatin was prepared by the method of Rajalakshmi et al.[13] The DNA was isolated after deproteinization of the chromatin with autodigested pronase (100 μg/ml) in the presence of 0.01 M Tris-HCl, pH 7.6, 0.05% sodium dodecyl sulfate and 1 M NaCl at 37 C for 4–5 hours and purified by Marmur's procedure.[14] The purified DNA was dissolved in 0.01 M NaCl and hydrolized in the presence of perchloric acid, 0.1 N final concentration, at 70 C for 45 minutes. Suitable aliquots were taken for monitoring absorption at A_{260}nm and radioactivity.

Serum GPT Determination

The serum activity of glutamate-pyruvate transaminase (GPT) was determined according to a standard combination method provided by Boehringer (Mannheim, West Germany).

Histologic Examination and Measurement of Liver Mitoses

Immediately after sacrifice of the animals, samples of liver tissue were fixed in 10% formalin, dehydrated, embedded in paraffin, and sectioned at 5 μ. De-

Table 1—Effect of Lead Nitrate on Body Weight, Liver Weight, and Hepatic DNA Content

Treatment* (number of injections)	Body weight		Relative liver weight (g/100 g body weight)		DNA (mg/g)		DNA (mg/100 g body weight)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	214.61 ± 10.89†	201.52 ± 6.70	4.52 ± 0.15	5.42 ± 0.14‡	2.41 ± 0.07	2.67 ± 0.09	11.08 ± 0.49	14.39 ± 0.65§
4	289.33 ± 7.34	275.76 ± 6.86	3.67 ± 0.06	5.08 ± 0.13‡	2.27 ± 0.11	2.39 ± 0.11	8.33 ± 0.44	12.20 ± 0.66‡
9	313.24 ± 20.84	301.14 ± 13.49	3.31 ± 0.07	4.21 ± 0.08‡	2.34 ± 0.14	2.48 ± 0.10	7.68 ± 0.33	10.49 ± 0.54‡

* Lead nitrate was injected intravenously at a dose of 5 μmol/100 g body weight once every 10 days. The animals were sacrificed 36 hours after the last injection of the metal. Controls received distilled water; 6–10 rats per group.

† Values are mean ± SE.

‡ Significantly different from control; $P < 0.001$.

§ Significantly different from control; $P < 0.005$.

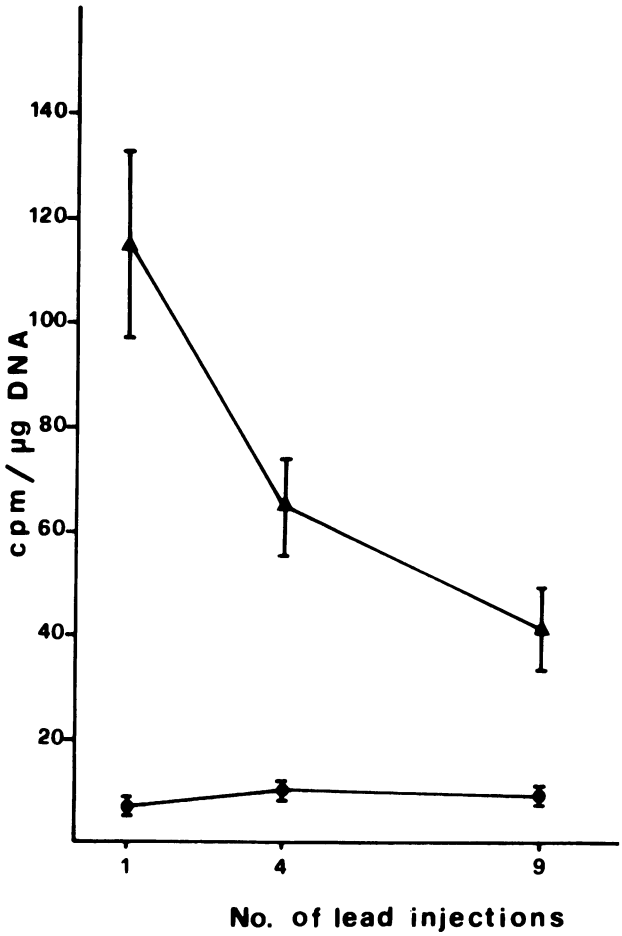

Figure 1—Time course of lead-induced changes in the incorporation of ^{3}H-thymidine into hepatic DNA. The mean ± (SE) represents at least 6–10 animals in each group. For details concerning treatment, see the legend to Table 1. The rats were killed 60 minutes after an intraperitoneal injection of 50 µCi/100 g body weight of ^{3}H-thymidine (sp. act. 25 Ci/mmole). ●——●, control; ▲——▲, lead-treated.

paraffinized sections were stained with hematoxylin and eosin.

For the mitotic index, expressed as number of mitotic figures per 100 nuclei, at least 15,000 nuclei were scored for each time point.

Results

Preliminary studies carried out in our laboratory indicate that a single dose of 5 µmol/100 g body weight of lead nitrate is able to induce DNA synthesis, as measured by the incorporation of labeled thymidine into hepatic DNA. Maximal activity of DNA was reached 30–36 hours after lead treatment; an increase in liver mass and total hepatic content of DNA at 2–3 days after treatment, with a return to normal values at 7–8 days after the administration of the metal, was also observed (data not shown).

Liver Enlargement

All the animals survived during the experimental period of 80 days. Percentage weight gain during the interval between the first injection of lead and sacrifice at 30 and 80 days was not significantly different from that of control rats (Table 1); the ratio of liver weight to body weight decreased in all animals, as a function of the age. The administration of lead caused a marked increase in the relative liver weight (20–40%), when compared with controls, at all time points examined.

The lead-induced enhancement in liver weight was accompanied by a significant increase in total hepatic DNA (30–40%) in all groups. A concomitant increase in protein content was also observed (data not shown).

Effect of Lead on the Incorporation of ^{3}H-Thymidine Into Hepatic DNA

The capacity of lead to induce DNA synthesis, as measured by the incorporation of ^{3}H-thymidine, is illustrated in Figure 1. The amount of radioactivity present in the DNA was significantly higher in rats treated with lead in all experimental groups. However, whereas a single treatment with lead induced a 16-fold increase in the specific activity of DNA, a progressive decline was observed following four and nine injections of the metal—a 6-fold and a 4-fold increase, respectively. In order to evaluate the possibility that the decrease in lead-induced thymidine incorporation after repeated injections could be due to the aging of the animals, 2- and 5-month-old rats, the latter having the same age as the animals treated with nine injections of lead, were given a single administration of the metal. The results (Table 2) indicate that 5-month-old rats receiving a single dose of lead incorporate labeled thymidine into DNA to the same extent as 2-month-old rats, thus suggesting that the decrease in the specific activity of DNA observed in Groups 2 and 3 was not age-related.

Table 2—Lead-induced ^{3}H-Thymidine Incorporation Into Hepatic DNA of Rats of Different Ages

Age* (months)	Number of animals	Incorporation of ^{3}H-thymidine (cpm/µg DNA)†
2	9	115 ± 18‡
5	9	124 ± 21

* Lead nitrate was injected intravenously at a dose of 5 µmol/100 g body weight, and the animals were sacrificed 36 hours afterwards.

† ^{3}H-Thymidine (50 µCi/100 g body weight) was injected intraperitoneally 1 hour before sacrifice.

‡ Mean ± SE.

Table 3—Effect of Lead Nitrate on Mitotic Index

Treatment (number of injections)	Mitotic index (number of mitoses/100 nuclei)
Control	0.04
Lead Nitrate	
1	4.14
4	0.99
9	0.16

Animals were sacrificed 36 hours after lead administration following one, four, and nine injections. At least 15,000 nuclei were scored for each time point. Groups of 8–10 rats were used.

Effect of Lead on Liver Cell Mitosis and Serum GPT

Mitotic activity in the hepatic cells of lead-treated rats followed a pattern similar to that seen with thymidine incorporation (Table 3). A mitotic index of 4.14, 0.99, and 0.16 was observed after 1, 4, and 9 injections of lead, respectively. All stages of mitosis were present (see Figure 2). Of liver cells in mitosis, 0.04% were found in the control group.

In order to determine whether the cell proliferation induced by repeated treatments with lead could be a consequence of lead-induced cell necrosis, the values of serum GPT were measured at various time intervals. The enzyme activity was found unchanged (26.5 ± 2.8 versus 19.1 ± 0.9 mU/ml of the controls after 30 days; 25.1 ± 2.7 versus 19.6 ± 1.9 after 80 days).

These results indicate that the increase in specific activity of DNA observed in all groups treated with lead is not due to a regenerative hyperplasia following necrosis, but rather to an adaptive response of the hepatocytes to lead.

Histologic Observations

The absence of cell death was confirmed by histologic examination. The liver of lead-treated rats showed nuclei of the hepatocytes often enlarged and with many nucleoli. An increase of the nuclear/cytoplasmic ratio was frequently seen. Liver sections of animals treated with nine injections of lead showed an increase in the number of binucleated cells; occasionally, cells with three nuclei could also be observed. Nuclei irregular in shape and vacuoles were present in this group.

Discussion

We have previously reported that a single dose of lead nitrate (10 μmol/100 g body weight) stimulates DNA synthesis in rat liver without evidence of cell

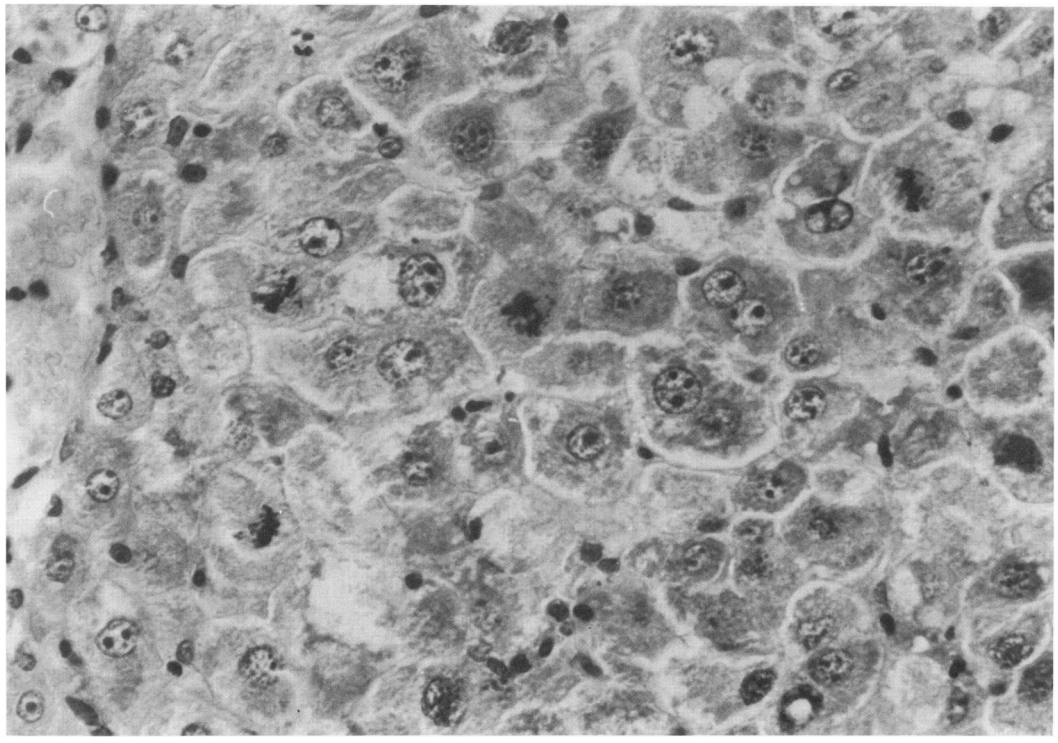

Figure 2—Liver of rat given four injections of lead nitrate and sacrificed 36 hours after the last administration. Mitotic figures are present. Enlarged nuclei with many nucleoli could also be observed. (×400)

death.[11] The results of this study indicate that proliferation of liver cells occurs also in rats receiving repeated injections of the heavy metal. The increase in the specific activity of DNA, after ^{3}H-thymidine incorporation, was never associated with any sign of cell necrosis even at 80 days after the initiation of the experiment, as shown by biochemical as well as histologic examination. The presence of mitotic figures in all the lead-treated groups would seem to indicate that the incorporation of labeled thymidine is not simply due to unscheduled DNA synthesis following DNA damage by lead. These facts suggest that in acute as well as during chronic treatment the lead-induced cell proliferation could be due to an adaptive mechanism rather than a toxic response.

Another interesting finding of this study was that even though the liver maintained its capacity to proliferate following repeated injections of lead, there was a steady decline of the magnitude of this response: the incorporation of labeled thymidine into DNA (see Figure 1), as well as the number of cell mitoses, maximal after the first treatment, are reduced after four administrations and further diminished after nine injections. Such reduced capacity to proliferate is not due to the older age of the rats, because animals of the same age as those receiving the ninth injection, treated with a single dose of lead, showed the same ability to incorporate thymidine as the younger animals.

The diminished capacity of lead to induce cell proliferation after repeated administrations is not unique to liver; indeed, the mean labeling index of renal cells was found to be 40 times that of cells in control rats after a single dose of lead acetate[8] but only 15 times that after repeated injections of the metal for six months.[9] As far as the proliferative capacity of hepatocytes is concerned, a partial loss has also been shown under different experimental conditions. A diet devoid of choline induces an intense proliferative response in the liver of rats after the first week, as monitored by the incorporation of labeled thymidine into DNA. Such an effect was already reduced after 2 weeks of continuous administration of the diet.[15] Thus, it would seem that the proliferative capacity of cells is diminishing after repeated applications of mitogenic stimuli. The biochemical mechanism(s) underlying these processes need to be fully elucidated; they are the same as those generally related to mammalian cell replication and are, to date, not well understood. For this reason it is even more difficult to understand the modification of cell growth occurring during the application of repeated mitogenic stimuli. Recently, Schulte-Hermann et al[16] showed that a surplus of DNA caused by α-hexachlorocyclohexane, an inducer of liver growth, is responsible for the decrease in DNA replication following a renewed treatment. It was concluded that the DNA content of the liver is controlled by a feedback system that monitors an excess of DNA and suppresses cell replication if the content of DNA exceeds normal levels. The possibility that repeated injections of lead could result in an increased DNA content, which in turn can reduce the response of the liver to successive stimuli by lead, is currently under investigation.

The biologic significance of cell proliferation has been put forward by Choie and Richter[9] in relation to lead-induced renal carcinogenesis. The results of our study on the liver are comparable to those reported by these authors on the effect of repeated injections of lead acetate on kidney cell proliferation in female rats.

Cell proliferation, in relation to carcinogenesis, has been considered to be an important contributing factor also in the liver, although the site and mechanism of its action has not yet been clearly defined. Pound et al. have shown that repeated injections of a necrogenic dose of carbon tetrachloride[17] or repeated partial hepatectomies[18] have a promoting effect on the appearance of neoplastic liver nodules in rodents receiving a single dose of nitrosamines; compensatory cell proliferation following necrosis, induced by carbon tetrachloride, also enhances the carcinogenic effect of a single exposure to ultraviolet light.[19]

In view of these considerations, the hyperplastic effect exerted by repeated administrations of lead in the absence of cell death may possibly offer another model for studying the association between cell proliferation and liver cancer.

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