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Influence of Lead and Cadmium on the Susceptibility of Rats to Bacterial Challenge (39117)

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Previous studies have demonstrated that certain heavy metals, particularly lead, when administered intravenously at sublethal doses to a variety of animals, markedly potentiate their susceptibility to bacterial endotoxins (1–4). The environmental contaminant cadmium has also recently been demonstrated to enhance lethality of rats to endotoxin (5). The mechanism by which lead or cadmium alter host response to bacterial endotoxin has not been fully clarified; however, alterations of the reticuloendothelial system (RES) and hepatic function have been predicated (5–7).

Although susceptibility to endotoxin is clearly compromised by acute exposure to lead, few studies have been conducted currently relative to the influence of lead on susceptibility to bacterial infection, while the influence of cadmium on host defense against bacteria has not been previously investigated. The present studies were therefore initiated to evaluate the effects of lead and cadmium on susceptibility of rats to bacterial challenge. Subsequent studies were conducted to determine if methylprednisolone, an agent demonstrated to prevent lethal lead–endotoxin interaction (8), would also modify lethality in lead- or cadmium-treated rats challenged with *Escherichia coli*. Since marked changes in hepatic morphology have been observed following the interaction of lead or cadmium with endotoxin (9, 10), light microscopic studies of the livers of rats following conjoint administration of lead or cadmium with *E. coli* were also undertaken.

Materials and methods. Male Charles River rats, weighing 180–200 g, were maintained on Purina Laboratory Chow and tap water *ad libitum*. Lead acetate was prepared in sterile 5% dextrose and administered 2.00 mg/100 g. Cadmium acetate was also prepared in 5% dextrose and slowly administered intravenously at a dose of 0.60 mg/100 g. Methylprednisolone (Solu medrol) was administered by intravenous injection at a concentration of 4 mg/100 g. All injections, including *E. coli*, were administered concurrently via the dorsal vein of the penis. Control groups were administered isovolumetric doses of 5% dextrose. The employment of the Limulus coagulation test (11) for detection of endotoxin indicated that the 5% dextrose and 0.9% saline were free of endotoxin.

Escherichia coli, 014, or *Staphylococci epidermidis* were maintained in Bacto Nutrient Broth (8.0 mg/ml) (Difco) at 37°C in a Dubnoff metabolic shaker (40 cycles/min) for 5 hr. The liquid culture was subsequently transfered to sterile tubes and centrifuged for 10 min at 800*g*. The bacterial pellet was washed and suspended with 5% dextrose, and the concentration of the bacteria was calculated immediately prior to injections.

Mortality data were analyzed by employing the chi-square test. All data were compared for statistical significance at the 95% confidence level.

For light microscopic studies, livers were dissected from rats and slices 0.5 cm in thickness were fixed in 4% buffered formalin. Tissues were dehydrated in graded series of ethanol and embedded in paraffin. Sections 6 μm in thickness were stained with hematoxylin and eosin.

Results. Normal rats displayed a significant tolerance to bacteremic episodes with the Gram-negative microbe (Table I). Administration of *E. coli*, 1×10^8/100 g, was nonlethal while injection of 10 times this amount of bacteria (1×10^9/100 g) resulted in a 13% mortality. Administration of 5×10^9 g *E. coli* was required before 50% of the rats eventually succumbed to the bacterial challenge.

TABLE I. EFFECT OF AN ACUTE DOSE OF LEAD ACETATE ON THE SUSCEPTIBILITY OF RATS TO *E. COLI*[a]

Group	Dose (*E. coli*/100 g)	Deaths/ total
Control	5×10^9	5/10
	1×10^9	1/8
	1×10^8	0/8
Lead acetate	—	0/10
	1×10^8	24/25
	1×10^7	13/15
	5×10^6	8/9
	1×10^6	0/16

[a] Lead acetate was administered in a dose of 2 mg/100 g. All injections were given intravenously and simultaneously with appropriate dilutions of *E. coli*. Control groups administered isovolumetric doses of 5% dextrose.

TABLE II. EFFECT OF AN ACUTE DOSE OF CADMIUM ACETATE ON THE SUSCEPTIBILITY OF RATS TO *E. COLI*[a]

Group	Dose (*E. coli*/100 g)	Deaths/ total
Control	5×10^9	5/10
	1×10^9	1/8
	1×10^8	0/8
Cadmium acetate	—	3/27
	1×10^8	23/24
	1×10^7	9/12
	1×10^6	6/15
	1×10^5	0/8
	1×10^3	0/5

[a] Cadmium acetate was administered in a dose of 0.6 mg/100 g. All injections were given intravenously and simultaneously with appropriate dilutions of *E. coli*. Control groups administered isovolumetric doses of 5% dextrose.

In contrast to the relatively innocuous effect of the bacteria in normal rats, doses of bacteria as low as 5×10^6 *E. coli*/100 g resulted in an 89% mortality in the acute lead-treated rats (Table I). The administration of lead acetate thus enhanced the lethal action of the microbes by at least 1000-fold. Acute exposure of cadmium acetate, like lead acetate, resulted in a marked increase in lethality when the rats were challenged with doses of bacteria that were otherwise readily tolerated in normal rats (Table II). A superimposed bacteremia of 1×10^6 *E. coli*/100 g resulted in a mortality of 40% in cadmium-treated rats, thus denoting a change in susceptibility to the bacteria in the range of three orders of magnitude.

Since the potentiating effect of lead or cadmium on the bacterial-induced lethalty might be due to the lipopolysaccharide component of the bacteria, similar experiments were conducted with heat-killed cultures of *E. coli*. Appropriate dilutions of *E. coli* were killed by autoclaving prior to intravenous administration. Doses of killed *E. coli* (1×10^8/100 g) were lethal to both lead- and cadmium-treated rats. The incidence of mortality in these groups, however, did not vary significantly from the mortality pattern of rats administered an equivalent dose of *E.*

TABLE III. SUSCEPTIBILITY OF LEAD- OR CADMIUM-TREATED RATS TO *E. COLI* FROM VIABLE OR NONVIABLE CULTURES, AND THE PROTECTIVE EFFECT OF METHYLPREDNISOLONE[a]

Treatment	*E. coli*		Lead acetate	Cadmium acetate	Deaths/ total
	Viable cultures	Nonviable[b] cultures			
Saline	+	−	+	−	24/25
	+	−	−	+	23/24
	−	+	+	−	14/15
	−	+	−	+	13/15
Methylprednisolone	+	−	+	−	0/10
	+	−	−	+	12/14

[a] *E. coli* were administered 1×10^8/100 g; methylprednisolone, 4 mg/100 g; lead acetate, 2 mg/100 g; and cadmium acetate, 0.6 mg/100 g.

[b] *E. coli* killed by autoclaving.

TABLE IV. EFFECT OF AN ACUTE DOSE OF LEAD ACETATE ON THE SUSCEPTIBILITY TO STAPHYLOCOCCI EPIDERMIDIS

Group[a]	Dose (*Staph. epidermidis*/ 100 g)	Deaths/ total
Control	1×10^9	0/8
Lead acetate	1×10^9	8/10
Lead acetate	1×10^8	0/10

[a] Lead acetate was administered at a dose of 2 mg/100 g. All injections were administered intravenously and simultaneously with appropriate dilutions of *Staph. epidermidis*. Control groups were administered isovolumetric doses of 5% dextrose.

coli (1×10^8/100 g) from viable cultures in the presence of lead or cadmium acetate (Table III). These observations, therefore, suggest that the primary toxic constituent of the Gram-negative bacteria is due to the heat stable endotoxin component. Furthermore, administration of equivalent doses (1×10^8/100 g) of viable cultures of the Gram-positive bacteria, *Staphylococci epidermidis*, failed to produce mortality in the lead-sensitized rats (Table IV). Higher doses of the Gram-positive bacteria (1×10^9/100 g), however, were lethal when administered in the presence of lead acetate ($p < 0.001$).

Since the synthetic glucocorticoid, methylprednisolone, abolished the synergistic toxicity of lead–endotoxin interaction (8), subsequent experiments were conducted to determine if this agent would exert significant protection in lead-treated rats in the presence of *E. coli*. Concurrent intravenous injection of methylprednisolone in rats exposed to lead acetate and *E. coli* (1×10^8/100 g), a dose which otherwise produced a 96% mortality, completely abolished the lethal interaction of the metallic salt with the bacteria ($p < 0.001$) (Table III). In contrast, methylprednisolone did not alter cadmium–*E. coli*-induced lethality.

No observable light-microscopic alterations in hepatic structure were apparent 16 hr after administration of lead acetate or *E. coli* (5×10^6/100 g) alone. The injection of cadmium acetate, however, resulted in congestion and occasional necrosis of parenchymal cells. Following 16-hr postadministration of a much higher dose of *E. coli* (1×10^9/100 g), disorganization of the lobular platelets was observed (Fig. 1). The livers of rats that received lead acetate and *E. coli* (5×10^6/100 g) 16 hr earlier presented focal areas of inflammation. Necrosis and vacuolization of hepatocytes were observed immediately surrounding the inflammatory area (Fig. 2). Hepatic lesions 16 hr following administration of cadmium acetate and *E. coli* (5×10^6) presented areas of pericentral and midzonal necrosis with significant inflammatory cell infiltrate (Fig. 3).

Discussion. The ability of the host to tolerate bacterial challenge is clearly compromised by acute exposure to lead or cadmium. Administration of lead acetate or cadmium acetate enhanced the susceptibility of rats to *E. coli* challenge by approximately 1000-fold. These data extend the studies of Hemphill *et al.* (12), in which chronic intraperitoneal injection of mice with lead enhanced lethality to Gram-negative microbe *Salmonella typhimurium*. Selye *et al.* (1) also demonstrated enhanced lethality of lead-sensitized rats to intravenous injection of 1% mixed fecal flora.

The current studies denote that the marked increase in susceptibility to Gram-negative bacteria is probably due to the lipopolysaccharide component. This contention is supported by our findings that (1) *E. coli* from heat-killed cultures produced a similar mortality in the presence of lead or cadmium as *E. coli* from viable cultures; (2) methylprednisolone prevented lethal interaction of lead with *E. coli* but did not significantly alter the mortality pattern in cadmium-*E. coli* treated rats, which agrees with previous studies of the effect of this glucocorticoid on mortality of lead or cadmium rats sensitized to endotoxin (5); and (3) mortality was not evident in lead-sensitized rats when an equivalent dose of Gram-positive bacteria was administered.

In conjunction with the ability of an acute dose of lead and cadmium to modify host susceptibility to bacteremic episodes and endotoxin, Gainer (13) demonstrated increased infectivity with Rauscher leukemic virus in lead-treated mice. Cadmium was also suspected of potentiating infectivity of the virus. In subsequent experiments, Gainer (14) demonstrated that chronic exposure of mice to lead increased their mortality upon exposure to a variety of viruses including Encephalomyocarditis, Pseudorabies, and Rauscher leukemia virus. The increased susceptibility could be due in part to alteration in immune status.

Koller (15) demonstrated a markedly depressed immune response to Pseudorabies virus in rabbits maintained chronically on drinking water containing lead acetate or cadmium acetate. A depressed immune response was also demonstrated in mice maintained for several weeks on lead-treated drinking water (16).

It is becoming increasingly evident that

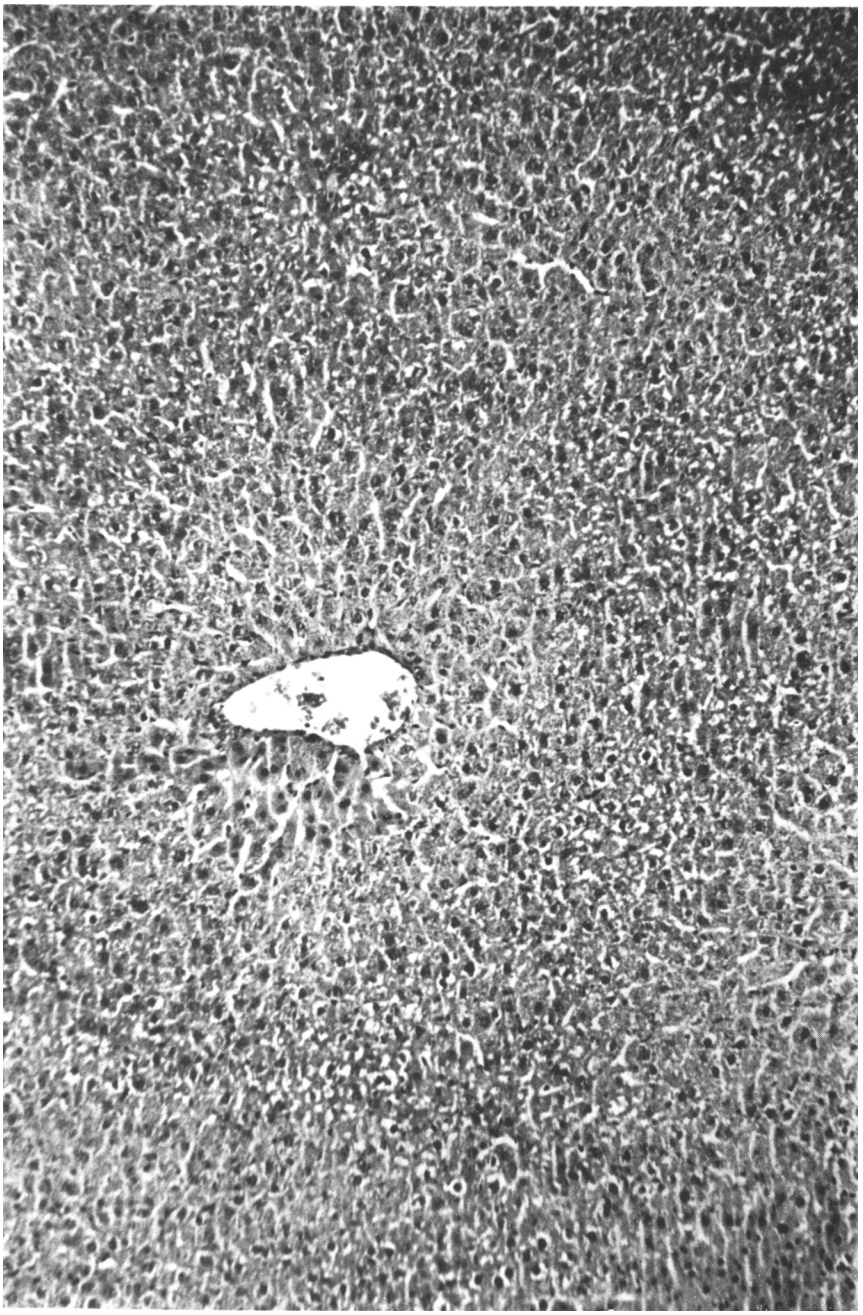

FIG. 1. The liver of a rat 16 hr after administration of *E. coli* (1×10^9/100 g). The liver presents disorganization of the lobular plates and occasional pyknosis of the nuclei of hepatocytes. ×200.

the pathological effects of certain metals may be related not only to the inherent toxicity of the metal itself but perhaps more importantly to conjoint events that transpire when they are present in the organism. While the acute doses of lead and cadmium employed in the current study may be far above the acute environmental level of exposure, long-term ex-

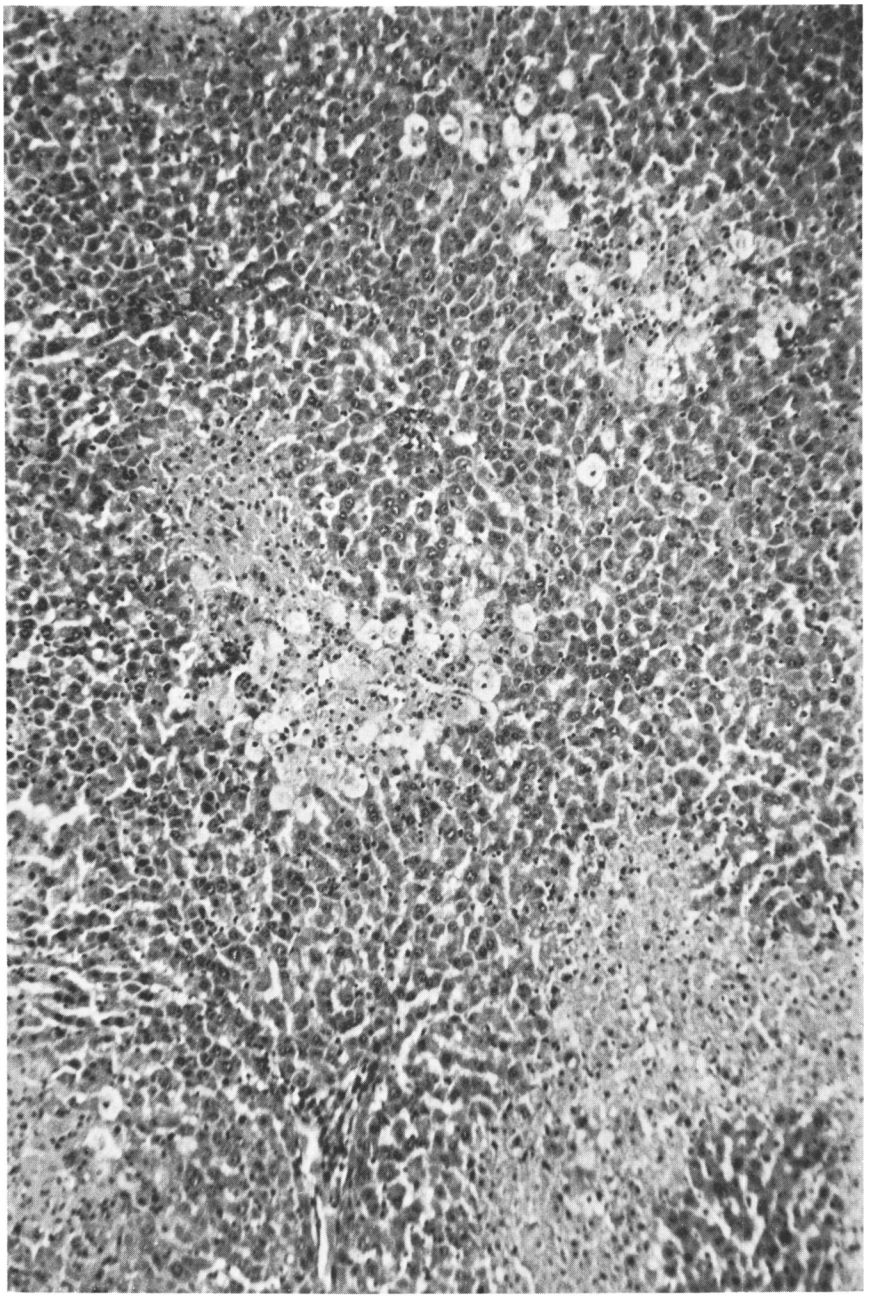

FIG. 3. Alterations in hepatic morphology following the administration of cadmium acetate (0.6 mg/100 g) and *E. coli* (5×10^6/100 g). The liver presents areas of pericentral and midzonal lesions showing areas of necrosis with inflammatory cells.

Summary. Intravenous administration of an acute dose of lead acetate or cadmium acetate enhanced the susceptibility of rats to intravenous challenge with *E. coli* by approximately 1000-fold. Since equivalent vulnerability of lead- or cadmium-treated rats to killed *E. coli* was observed, toxicity is probably due to the endotoxin

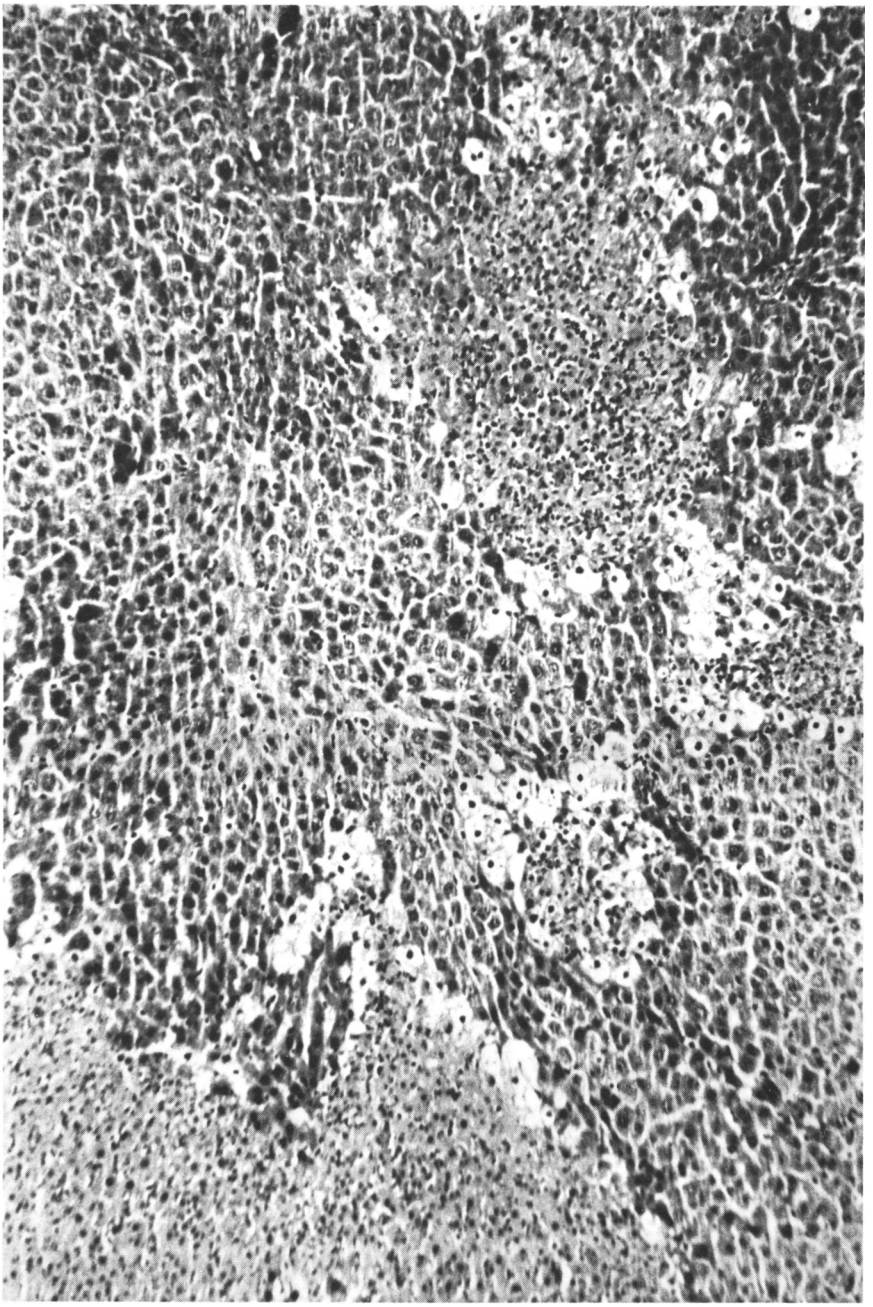

FIG. 2. The liver of a rat 16 hr following administration of lead acetate (2 mg/100 g) and *E. coli* (5×10^6/100 g). There is disorganization of hepatic integrity with inflammatory cells surrounded by vacuolated hepatocytes. The remainder of the liver appears slightly disorganized.

posure of animals to such agents have not been sufficiently investigated. In view of the increasing awareness of the diverse pathological effects of lead (17) and cadmium (18), the effect of these metals, as well as other environmental agents on host susceptibility to bacterial and viral infections warrants further investigation.

content of the bacteria. This postulate is further supported by the observation that equal doses of the Gram-positive bacteria, *Staphylococci epidermidis*, failed to elicit lethality in the acute lead-intoxicated rats. The synthetic glucocorticoid, methylprednisolone, prevented lethality induced by the Gram-negative bacteria in lead-treated rats. It did not, however, afford significant protection in cadmium-treated rats in the presence of *E. coli*. Marked alterations in hepatic morphology were apparent in both lead- and cadmium-treated rats challenged with *E. coli*.

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1. Selye, H., Tuchweber, B., and Bertok, L., *J. Bacteriol.* **91,** 884 (1966).
2. Holper, K., Trejo, R. A., Brettschneider, L., and Di Luzio, N. R., *Surg. Gynecol. Obstet.* **136,** 593 (1973).
3. Rippe, D. F., and Berry, L. J., *J. Reticuloendothel. Soc.* **13,** 527 (1973).
4. Truscott, R. B., *Can. J. Comp. Med.* **34,** 135 (1970).
5. Cook, J. A., Marconi, E. A., and Di Luzio, N. R., *Toxicol. Appl. Pharmacol.* **28,** 292 (1974).
6. Trejo, R. A., Di Luzio, N. R., and Hoffmann, E. O., *Exp. Mol. Pathol.* **17,** 145 (1972).
7. Filkins, J. P., *Proc. Soc. Exp. Biol. Med.* **142,** 915 (1973).
8. Cook, J. A., and Di Luzio, N. R., *Exp. Mol. Pathol.* **19,** 217 (1973).
9. Hoffmann, E. O., Di Luzio, N. R., Holper, K., Brettschneider, L., and Coover, J., *Lab. Invest.* **30,** 311 (1974).
10. Cook, J. A., Di Luzio, N. R., and Hoffmann, E. O., *CRC Crit. Rev. Toxicol.* 201–229 (1975).
11. Rojas-Corona, R. R., Skarnes, R., Tamakuma, S., and Fine, J., *Proc. Soc. Exp. Biol. Med.* **132,** 599 (1969).
12. Hemphill, F. E., Kaeberle, M. L., and Buck, W. B., *Science* **172,** 1031 (1971).
13. Gainer, J. H., *J. Natl. Cancer Inst.* **51,** 609 (1973).
14. Gainer, J. H., *Environ. Health Perspect.* (Experimental issue) **7,** 113 (1974).
15. Koller, L. D., *Amer. J. Vet. Res.* **34,** 1457 (1973).
16. Koller, L. D., and Kovacic, S., *Nature* **250,** 148 (1974).
17. Goyer, R. A., and Rhyne, B. C., *in* "International Review of Experimental Pathology" Academic Press, New York. Vol. 12. (G. W. Reichter and M. A. Epstein, eds.), (1973).
18. Friberg, L., Piscator, M., and Nordberg, G., "Cadmium in the Environment." Chem. Rubber Co., Cleveland (1971).

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