tional experiment. A polyethylene tank 10 cm deep and 200 cm in diameter was shielded from direct sunlight, filled with distilled water, and exposed to the air. Radium was added, and the turbulent conditions were varied by changing the rate at which water was recirculated within the tank until the rate of radon loss yielded a piston velocity equal to that observed for Lake 227. Potassium hydroxide was then added, bringing the *p*H to approximately 10 (that of the Lake 227 epilimnion). The change in CO_2 content with time was monitored. The rate of CO_2 invasion was found to be between three and four times that expected in the absence of any chemical enhancement for these physical conditions (*11*). Hence, the enhancement factor calculated from the tank experiment agrees satisfactorily with that calculated from the elemental balance data for Lake 227.

We conclude that invasion of CO_2 from the atmosphere supplies sufficient carbon to Lake 227 to produce an algal bloom in proportion to the amount of phosphorus and nitrogen added. Since the lake had an unusually low natural carbon content and is extremely well protected from wind, we further conclude that invasion of CO_2 from the atmosphere is likely to be sufficient for the eutrophication of any lake receiving sufficient supplies of nitrogen and phosphorus.

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5. D. W. Schindler, V. E. Frost, and R. V. Schmidt (unpublished results) have found that in similar lakes the littoral zone contributes less than 5 percent to the total annual primary productivity.
6. D. R. S. Lean and D. W. Schindler (unpublished results) have studied movement of ^{14}C in labeled sediments and water columns for a period of 1 year. Less than 1 percent of the carbon that falls to the sediments and hypolimnion from the epilimnion ever reaches the euphotic zone of the lake again.
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9. The detailed results of the radon experiment and an explanation of the method used to calculate these values are being prepared for publication by S. Emerson.
10. The relationship used here between the flux of a gas across the atmosphere-lake interface and the piston velocity is:

$$F = (C^{\text{lake}} - P^{\text{atm}}\alpha)V$$

where F is the flux of the gas (moles per square meter), C is the concentration of the gas in the water (moles per cubic meter), P is the partial pressure of the gas in the atmosphere (atmospheres), α is the solubility of the gas in water (moles per cubic meter), and V is the piston velocity (meters per day). Since V has units of distance over time it may be written as the diffusion constant D for radon (that is, 1.36×10^{-5} cm²/sec), divided by a distance term Z, which has been described as representing "a stagnant boundary layer" on the water surface (*11*) and is used in models for gas exchange rates. Once Z is determined by radon measurements, F may be estimated for CO_2 gas because P, α, and D are known for that gas (here we have used 1.92×10^{-5} cm²/sec as the molecular diffusion constant for CO_2).
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Lead Poisoning: Rapid Formation of Intranuclear Inclusions

Abstract. *A single dose of lead (0.05 milligram per gram of body weight) induced characteristic intranuclear inclusions in the epithelium of proximal tubules in rat kidney within 1 to 6 days. The development of the intranuclear inclusions is thus an acute manifestation of lead poisoning, not a delayed one, as has been thought hitherto. Cytoplasmic structures resembling the intranuclear inclusions and situated in the vicinity of endoplasmic reticulum were regularly found in cells bearing the pathognomonic intranuclear inclusions. The latter and the cytoplasmic structures may be derived from a common precursor, perhaps a soluble protein-lead complex.*

In man and in various animals the pathognomonic morphological feature of chronic intoxication with lead is the presence of characteristic intranuclear inclusions in proximal tubular epithelial cells of the kidneys (*1*). The inclusion bodies are eosinophilic and do not appear to contain DNA or RNA but do contain protein, presumably of nonhistone type (*2*). Chemical analysis of inclusions separated from cell homogenates by centrifugation has indicated the presence of lead as well as of protein in the inclusions (*3*). The development of these intranuclear inclusions in proximal tubular epithelial cells has generally been connected with chronic lead poisoning and has been thought to require 4 to 8 weeks of exposure to lead (*1–3*). We have found, however, that in rat kidneys typical intranuclear inclusions occur as early as 24 hours after a single intraperitoneal injection of lead. This indicates that the development of the pathognomonic intranuclear inclusions in renal tubular epithelium is an acute manifestation of lead poisoning.

Young adult female Sprague-Dawley rats, weighing 220 to 270 g, were injected intraperitoneally with lead acetate in sterile water, in doses of 0.05 to 0.20 mg of lead per gram of body weight. From 1 to 6 days after the single dose of lead, tissue was taken from the cortex of each kidney, fixed for 3 hours in 2 percent glutaraldehyde in Millonig's (*4*) buffer (*p*H 7.35) and then for 1 hour in 1 percent osmium tetroxide in Millonig's buffer, and subsequently embedded in Epon 812 epoxy resin (*5*). Thin sections were prepared for electron microscopy and were examined with a Siemens Elmiskop 101 at 80 kv after staining with Reynolds' stain (*6*) or after staining with saturated aqueous uranyl acetate followed by Reynolds' stain.

Results of a survey of kidneys from 38 rats are shown in Table 1. Intranuclear inclusions were detected in 10 to 25 percent of epithelial cells in proximal tubules as early as 1 day after the injection of lead. These inclusions were found only in the epithelium of proximal tubules and not elsewhere in the nephron. The percentage of proximal tubular cells with intranuclear inclusions during the 6-day period was not significantly changed by increasing the dose of lead. As seen by electron microscopy of thin sections, most of

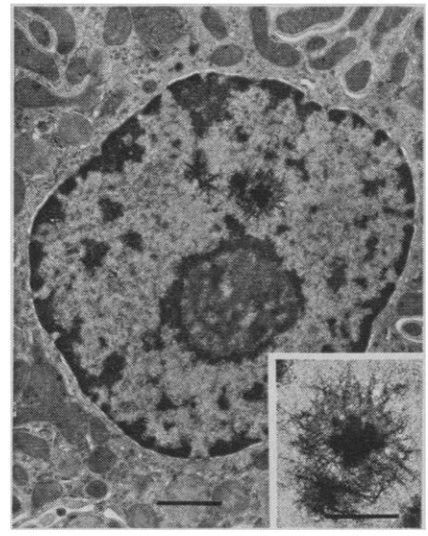
the intranuclear inclusions consisted of a characteristic fibrillar mesh and of dense amorphous material, the latter usually located in the interior of inclusions (Fig. 1). The fibrils varied in length (50 to 250 nm) and measured approximately 120 Å in thickness.

Sometimes one nucleus contained two or more "lead" inclusions. In relation to the nuclear volume, the rapidly formed lead inclusions were smaller in size than inclusions found in rats or in man after prolonged exposure to lead. In this early phase of lead poisoning, cytoplasmic organelles appeared normal.

Because of the possibility of accidental ingestion of lead prior to the experimental period, kidneys from 15 untreated rats in the same colonies were also scrutinized. Intranuclear inclusions were never found in kidneys from these controls.

It must be noted that in all but two rats injected with lead acetate, some lead salt (or oxide) was precipitated in the peritoneal cavity, indicating that the actual amount of lead absorbed was less than the dose injected.

The origin of the intranuclear inclusions associated with lead poisoning has yet to be determined. The protein component may be either derived from a preexisting nucleoprotein or may be synthesized de novo. Müller and Stöcker (*7*) could not detect incorporation of [^{14}C]phenylalanine into intranuclear inclusions in rats chronically treated with lead, and Richter *et al.* (*2*) proposed that these inclusions result from degradation and restructuring of a nonhistone nucleoprotein. However, synthesis of the protein component de novo has not been ruled out. For example, it is possible that in response to the administration of lead, a protein is synthesized in the cytoplasm, bound to lead, transported into the nucleus, and there polymerized into the characteristic fibrils of the inclusions. In accord with this possibility, we have observed distinct cytoplasmic structures that resemble the pathognomonic intranuclear inclusions (Fig. 2). These cytoplasmic structures were composed of fibrillar and amorphous material and were found only in proximal tubular epithelial cells of leaded rats. They were located in regions of endoplasmic reticulum, and were visible by electron microscopy even in unstained sections. Interestingly, the presence of these cytoplasmic structures coincided with that of the intranuclear inclusions. Both may be derived from a common precursor, perhaps a soluble complex of protein and lead.



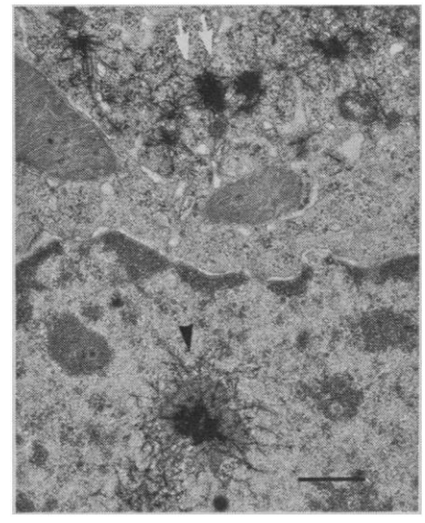


Fig. 1 (left). Electron micrograph of part of epithelial cell in proximal tubule of rat kidney, showing characteristic intranuclear inclusions above a nucleolus. Tissue was taken 4 days after a single intraperitoneal dose of 0.1 mg of lead per gram of body weight. Stained with uranyl acetate and Reynold's lead stain (scale, 1 μm). Inset shows structural details of intranuclear inclusion (scale, 0.5 μm). Fig. 2 (right). Electron micrograph of part of proximal tubular epithelial cell from a rat, 4 days after a single intraperitoneal dose of 0.15 mg of lead per gram of body weight. Note cytoplasmic structures (white arrows), located in area of rough endoplasmic reticulum, that resemble the intranuclear inclusion (black arrowhead). Stained with uranyl acetate and Reynolds' lead stain (scale, 0.5 μm).

It seems likely that further work on the nature and genesis of rapidly induced lead inclusions will help to clarify the pathological effects of lead. We have reported previously that a single dose of lead stimulates DNA synthesis in rat kidneys within 2 days (*8*). One may ask, therefore, whether the formation of the intranuclear inclusions in renal tubular cells is in any way related to the stimulation of nuclear DNA synthesis in these cells.

The rapid appearance of intranuclear inclusions may be useful as an early morphological sign of acute lead poisoning in man, if renal biopsy specimens are examined by electron microscopy.

Table 1. Incidence of intranuclear inclusions in epithelial cells of proximal tubules of rat kidneys 1 to 6 days after injection of lead.

Days after injection of lead	Dose of lead (mg/g body weight)	Rats with intranuclear inclusions*
1	0.05	3/3
	.1	3/3
	.15	2/3
2	.1	3/3
	.15	5/5
3	.1	2/3
	.15	3/3
4	.1	5/5
	.15	4/4
5	.15	3/3
6	.2	3/3

* Number of rats with intranuclear inclusions in proximal tubular epithelial cells per number of rats examined.

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